RESPONSE OF CHICKPEA CULTIVARS TO DRYLAND MANAGEMENT IN SOUTHERN ETHIOPIA

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Department of Plant Sciences, University of Saskatchewan, Saskatoon, Saskatchewan

By

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is a popular and profitable pulse crop for the southern Ethiopian region, yet, due to the lack of improved production technology the average yield of chickpea in the region is relatively low. The current research assessed the agronomic performance of chickpea cultivars as affected by seeding dates and rhizobium inoculation across agro-ecologies in southern Ethiopia. The research also examined the effect of residual nitrogen on wheat grain yield in chickpea wheat rotation. The impact of soil water deficit on nitrogen fixation and seed composition of a set of chickpea cultivars was evaluated under greenhouse conditions.

Seeding date can be used as a strategy to avoid high temperatures during flowering and to reduce the effect of water deficit during pod filling. The yield and agronomic characteristics of the chickpea cultivars varied with different seeding dates. The Habru and Ejere cultivars when seeded at a mid or late seeding date tended to flower and mature early across all locations. This indicated that the plants were exposed to stress condition under late seeding resulting in a short growing period. The seeding date experiment showed a grain yield advantage under early seeding date. Growing chickpea under residual moisture immediately after the harvest of the main crop allows farmers to maximize the use of their land by double cropping.

Response of chickpea cultivars to *Rhizobium* inoculation confirmed that the environmental factors and the host symbiont compatibility greatly affected nitrogen fixation. Despite the low total soil nitrogen concentration and the low population (< 10 $\mu g\ g^{-1}$) of the native rhizobia at Wolaita, the inoculation treatment had no effect on seed yield in both 2011 and 2012 trials. This could be due to poor adaptation of the commercial strain of chickpea inoculant used in the study to the research area. It was observed that all the cultivars had small nodule number and the nodules were mostly ineffective. The chickpea cultivars had variable % Nitrogen derived from the atmosphere, ranging from 26 to 54%, regardless of inoculation treatment. The variation of % Nitrogen derived from the atmosphere was relatively high across environments.

The results of the analysis of soil samples taken from the seeded plot areas indicated that soil N concentration increased from 0.16% N, before chickpea was planted, to 0.24% N after chickpea was harvested possibly due to the decomposition of the chickpea residue. The rotational benefit of chickpea was evaluated by combining with management practice like application of low rate

of nitrogen fertilizer on wheat. The wheat yield grown with low rate nitrogen fertilizer after chickpea was 19 % higher than that grown on the non-fertilized plot after chickpea.

Variability of chickpea cultivars in response to water deficit was examined. The accumulation of ureide and nitrate in the shoot varied across chickpea cultivars under water stress condition. The ureide concentration in leaf tissue was twice or more than its concentration in the stem indicating fast translocation of assimilate towards the sink due to stress conditions. Moisture stress reduced stem ureide concentration by 37 % and increased stem nitrate concentration by 62 % compared to control. Concentrations of eighteen different amino acids were estimated in chickpea leaves. High proline, threonine and serine in leaves may have positive effects in osmoregulation as seen in ILC 533 and CDC Chico. Twelve free amino acids were estimated in seeds of 15 cultivars grown under water deficit. The water stress treatment decreased most free amino acid concentration in the majority of the cultivars, except CDC Chico.

In conclusion, increasing chickpea productivity on smallholder farms in southern Ethiopia is possible by using improved cultivars with appropriate agronomic practice, namely early seeding. *Rhizobium* inoculant research should test more strains (domestic or imported) to ascertain the best host-strain combination. The influence of water deficit on nitrogen fixation in chickpea indicated that cultivars varied in their accumulation of ureide and nitrate in the shoot. Screening of germplasm for improved N fixation under water stress should include measurement of leaf amino acids such as serine, proline, and threonine. Emphasis should be given to nodule and shoot ureide relationships, and root and stem nitrate concentrations during the stress period.

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1. Introduction

Chickpea (*Cicer arietinum* L.) is the world's second most important pulse crop after dry bean (Akibode and Maredia, 2011). Chickpea is grown widely in Ethiopia covering an area of 239,747 hectares with a total production of 458,682 tonnes in the 2014-2015 growing season (Central Statistics Authority of Ethiopia, 2015). In Ethiopia, chickpea serves several purposes such as a food, cash, and a soil fertility crop (Shiferaw *et al.*, 2007). In addition, chickpea is considered as a less labor-intensive crop, its production requires relatively lower inputs compared to cereals. Chickpea is mostly grown from September to December using residual moisture after the main season crop is harvested. Chickpea fixes atmospheric nitrogen (N), improves soil fertility, and saves fertilizer costs in the subsequent crops. These conditions allow more intensive and productive use of land.

There are two types of chickpea, namely desi and kabuli. Both kabuli and desi types generally have yellow cotyledons. Kabuli chickpea has a thin transparent seed coat, whereas the desi type has a thick, reddish brown-colored seed coat. Kabuli seeds are generally larger than desi seeds. The desi type is traditionally and most commonly produced in Ethiopia, whereas the kabuli type production is limited to a few pocket areas, where the use of improved cultivars has been promoted (Shiferaw and Hailemariam, 2007).

Chickpea helps to reduce malnutrition and improves human health especially for the poor who cannot afford animal protein. It is an excellent source of protein, fiber, complex carbohydrates, vitamins, and minerals. In addition to being a source of cash for smallholder producers, chickpea increases livestock productivity as the crop residue is rich in digestible crude protein compared to cereals (Menale *et al.*, 2009).

The soils of Ethiopia, especially those with frequent cereal cultivation, are generally deficient in N-fixing bacteria *(Rhizohium* spp.) which contribute to low yield of chickpea (Shiferaw and Hailemariam, 2007). There is potential to increase chickpea productivity by exploiting better colonization of the roots and rhizosphere through the application of effective nitrogen fixing bacteria to the seed or to the soil. This can minimize the need for inorganic nitrogen fertilizer which is costly in Ethiopia.

Low yield of chickpea in Ethiopia is mainly attributed to the lack of use of improved chickpea production technology. This was evident in 2014-2015 when all of the chickpea production area (239,747 ha) was managed by smallholder farmers, and of this area less than 25 % was seeded with improved cultivars. The rest of the production areas were seeded with local landraces (Million and Asnake, 2014). Although the Ethiopian average chickpea yield, at 1.7 tonnes ha^{-1} (Bulletin of Tropical Legumes 19, 2013) is higher than other countries including India, farm tests on experimental plots in Ethiopia have achieved yields from 2.9 to 3.5 tonnes ha^{-1} (International Food Policy Research Institute, 2010). This implies a productivity gap of at least 1.2 tonnes ha^{-1}. Bridging this gap would make Ethiopia among the major chickpea producing countries. Despite the development and release of a number of improved chickpea cultivars over the past three decades by the national breeding program, the potential of these cultivars has not been tested in the southern region of Ethiopia. This could be mainly attributed to resource limitation, lack of appropriate technology and to some extent less attention given to chickpea by research institutes in the southern region. Therefore, there is an opportunity to increase chickpea productivity per unit area in the region through introduction of appropriate and affordable technology practices that include optimum seeding date, inoculation, improved early and high yielding cultivars since most of the chickpea crops are grown using residual soil moisture.

The effects of soil water deficit on yield and yield characteristics of various pulse crops have been well documented. The studies also demonstrated how moisture stress affected biomass, height, flowering and seed development at different growth stages of the crop. However, research data are limited on the relationship of soil water deficit and nitrogen fixation at flowering in chickpea, and on how water stress affects the amino acids composition in plant organs and in seed. Chemical composition of seed, especially the concentration of carbohydrates, amino acids and proteins, has direct effect on the nutritive value of the crop (Behboudian *et al*., 2001). Dwiviedi *et al.* (1996) reported increased seed protein concentration in groundnut (*Arachis hypogaea* L.) under water stress. Increased concentration of proteins and amino acids such as leucine, valine, glycine and proline in common bean (*Phaseolus vulgaris*) under water stress was reported by Gyori *et al.* (1998).

In this thesis four research components were studied, which consisted of three field experiments conducted in 2011 and 2012 cropping seasons across three locations in southern Ethiopia.

Research component one addressed the appropriate seeding date of chickpea which could be a major factor in avoiding terminal drought. The use of local landrace and the seeding date commonly practiced by the local growers often exposed the crop to terminal drought leading to low yield. The introduction of improved cultivars with proper agronomic practice including seeding date is considered as the best intervention. The response of the improved chickpea cultivars to rhizobia inoculant was studied in research component two, which was conducted in the same locations to specifically address the problem of low soil nitrogen through increased nitrogen fixation. Component three was a continuation of the inoculation study in order to quantify the effect of residual soil nitrogen on the grain yield and straw yield of wheat (*Triticum aeasivum*) following chickpea. Component four was conducted in the greenhouse to study the effects of soil water deficit (drought), a common problem in chickpea production, on nitrogen fixation. In addition, component four determined the variability of drought tolerance among the selected genotypes. This research also quantified the effect of moisture stress on seed amino acid composition.

The four research components were conducted to test four general hypotheses.

1.1 Hypotheses

- Appropriate chickpea seeding date will maximize crop yield in each specific agro-ecological sites.
- Rhizobia inoculation improves the yield of chickpea cultivars.
- Growing wheat on rhizobium inoculated chickpea plots will produce higher yield than growing on non-inoculated plots in a chickpea-wheat rotation system.
- Significant variation in nitrogen fixation will be detected across chickpea cultivars under drought conditions and better fixing chickpea cultivars will produce greater seed yield.

1.2 Objectives

- To study the response of chickpea cultivars grown under different seeding dates and agro-ecological sites of southern Ethiopia.
- To evaluate the response of chickpea cultivars to rhizobia inoculation across agro-ecological sites of southern Ethiopia.

- To examine the effects of residual nitrogen from chickpea on wheat in legume-cereal rotation.
- To evaluate the differences in nitrogen fixation activity and seed composition of 15 chickpea cultivars under soil water deficit conditions.

2. Literature Review

2.1 Chickpea production status and challenges

In Ethiopia, chickpea is mainly grown in the central, northern and eastern highland areas of the country at an altitude of 1400-2300 m above sea level, where annual rainfall ranges between 700 and 2000 mm (Anbessa and Bejiga, 2002). The average productivity of Ethiopian chickpea in 2014/15 was 1.9 tonnes ha^{-1} (Central Statistics Authority, 2015) which is far from the 3 tonnes ha^{-1} that can be expected under favorable conditions.

Over the past 14 years there has been an increasing trend in the total area of production, the quantity of chickpea produced, and the overall productivity of chickpea in Ethiopia (Abate *et al*., 2011). During 2000-2013, Ethiopian chickpea harvested area, chickpea production and yield showed annual growth rate of 0.14 %, 7.16 % and 7.01 % respectively (FAOSTAT, 2014).

Ethiopia has suitable agro-climatic conditions for production of both desi and kabuli types of chickpea. The crop is highly integrated into the farming system and ecologically friendly for growing in many areas. Chickpea can be widely grown on different soil types provided there is good moisture and drainage. Well drained black soils, which have good water holding capacity, are suitable soil types for optimum growth of chickpea (Menale *et al*., 2009).

The Ethiopian Agricultural Research Organization (EARO) Chickpea Research Team has focused mainly on breeding and selection of cultivars with higher yield, disease resistance and drought tolerance. The EARO released 16 improved chickpea cultivars (7 kabuli types and 9 desi types) from 1974 to 2009 (Ministry of Agriculture and Rural Development, 2009).

However, this did not result in the desired level of productivity because the average yield calculated from 2000 to 2013 was still below 1.6 tonnes ha^{-1} (FAOSTAT, 2014). Cultivar development can be seen as a component of a technology package through which crop yield can be improved and it has to be supported by appropriate agronomic management including seeding date, land preparation, site selection, optimum fertilizer rate, proper weed control, rhizobium inoculation, and disease and pest control measures.

2.2 Effects of seeding date

Different seeding dates influence the vegetative and reproductive stages of the plant through variation in temperature, solar radiation and day length. Also, seeding date is important for chickpea that usually experiences dry conditions or relies on moisture stored in the soil to avoid terminal drought. Factors that are important in the selection of seeding date include climatic factors (rainfall, temperature, light and day length) and non-climatic factors, such as cultivar choice, pests, diseases and weed prevalence and seed bed preparation (Khajehpour, 2000).

Selection of the appropriate seeding date is crucial to maximize resources in a short growing season. Early seeding of seed in cold bed (< 10^0C) can cause poor establishment of plants and cold damage to plants and foliage may increase (Ozdemir, 1996). Delay in seeding causes shortening of growing seasons and increases the risk of drying of seed bed (Khajehpour, 2000).

Yield loss in chickpea can vary between 30 - 60 % depending on cultivar, seeding time, location, and climatic conditions during seeding season (Kabir *et al*., 2009). In areas with dry seed bed conditions some chickpea cultivars have the capacity to tolerate drought and in these cases seeding time can be delayed. However, earlier or late seeding has caused drastic reduction in yield compared to the yield from timely seeding (Dixit *et al.*, 1993). Ten breeding lines of chickpeas were evaluated for their response to seeding dates under two Mediterranean climates of Jordan. Late seeding (spring seeding) significantly lowered some seed yields at both locations (Al-Rifaee *et al.*, 2007). A study conducted to see the effect of seeding time and cultivars on the growth and yield performance of chickpea under rain-fed condition showed significant interaction difference between cultivar and seeding time in numbers of pods per plant, seed yield ha^{-1}, plant height, canopy coverage and harvest index, but showed no difference in numbers of seeds pod^{-1}, 100-seed weight and seed yield $plant^{-1}$ (Kabir *et al.*, 2009). Three seeding dates and three spacing were used to investigate the impacts of seeding date and row spacing on yield and yield components of Hashem chickpea cultivar. Results showed that there were significant effects of seeding date and seeding density on plant height, number of branches $plant^{-1}$, distance between first pod to soil surface, number of pods $plant^{-1}$, number of grains $plant^{-1}$, and grain yield (Shamsi, 2009).

In an experiment conducted to compare seeding date and plant density effects on yield and yield components of chickpea in Iran, seed yield was significantly affected by seeding dates, while the effect of plant density on the seed yield was not significant. Crops seeded in mid-March and mid-November produced highest seed yield (1042 and 963 kg ha^{-1} respectively), followed by mid-April (709 kg ha^{-1}) seeding. Results were due to the reproductive phase of the early seeding crop being initiated in a more favorable thermal and moisture regime than mid-April sown crops (Valimohammedi *et al*., 2007).

Kabir *et al*. (2009) reported that crop growth rate (CGR) increased gradually as chickpea grew from emergence to 75 days after emergence irrespective of cultivar, but declined afterwards. Seeding date influenced the crop growth rate variably, for early seeding post-flowering CGR was higher than pre-flowering CGR. Hussain *et al.* (1997) observed the comparative, superior performance of early seeding to late seeding in total dry matter production and explained that this might be due to a higher leaf area index (LAI).

2.3 Nitrogen fixation

Legumes are noted for their ability to fix atmospheric dinitrogen through symbiotic relationships with N-fixing bacteria (Winkler *et al*., 1988). Chickpea like most legumes establishes a symbiotic association with a compatible strain of rhizobium. Biological nitrogen fixation (BNF) is a process by which N_2 in the atmosphere is reduced into a biologically useful, combined form of ammonia-N by living organisms (Hardy and Burns, 1968; Giller, 2001). The greatest proportion of N found on the earth is located in the atmosphere, as N_2. Nevertheless, the majority of organisms cannot utilize this free and abundant, but highly stable gaseous source of N because they can only use N which is combined with other atoms into plant usable forms, such as ammonium, nitrates and ammonia (Giller, 2001; Giller and Cadisch, 1995). Most plants access their N by uptake of nitrate, ammonium, and even small amino acids (forest soils) by root uptake. The soil N source is determined by microbial activity via N immobilization and mineralization. The process of making gaseous N_2 available constitutes interaction of soil microbes (bacteria) and higher plants via the formation of nodules (Sessitsch *et al*., 2002). Nodules are formed on roots or, in some cases, stem (Tamimi and Timko, 2003).

Nitrogen fixation occurs inside the root nodules that form on legume as a result of symbiosis between the host plant and bacteria. There are two types of nodules on legumes: determinate and indeterminate (Streeter, 1991). Determinate nodules are spherical in shape with no meristem and the nodule tissue mature evenly. Indeterminate nodules are generally cylindrical in shape with a meristem, and nodule cells are continuously formed at the elongated nodule tip (Streeter, 1991). Chickpea has determinate nodules (also known as crown nodules) because they are located predominantly at the crown of the root system.

Rhizobia-legume symbiosis system involves different steps. Legume roots exude flavonoids that induce the expression of a set of rhizobial genes (Denarie *et al*., 1996) thus recognition of symbiotic partners; attachment of the rhizobia to the plant root hairs; root hair deformation; invasion of the root hair by rhizobia; infection thread formation; nodule initiation; bacteriod development; and formation of the N_2-fixing nodules (Hirsch, 1992; Mylona *et al*., 1995).

Once the symbiosis is established the host plant provides energy in the form of ATP and carbohydrate, and the bacteria produces nitrogenase enzyme to reduce atmospheric N_2 to ammonia, which is exported to plant tissues for protein synthesis (Keyser and Li, 1992; Paul and Clark, 1996). The plant benefits by assimilating the micro symbiont NH_3 to amino acids, namely amides (2N containing amino acids like asparagine and glutamine) and uriedes (4N containing cyclic amino compound). The amide and uriedes in warm-season N-fixing legumes are transported to the rest of the plant for later metabolic use (Peoples *et al*., 1985; Streeter, 1991; Sinclair and Seraj, 1995). The N_2 fixed by legume crops has an economic value, in terms of both the N itself and rotational benefits. The value of fixed N in a legume crop can be calculated using an average value for % Ndfa (the proportion of legume N derived from N_2 fixation) and the average yield data (IAEA, 1998).

Effects of chickpea on soil organic fertility are conflicting. Ibsa (2013) observed improvement in chickpea yield (2 tonnes ha^{-1}) after inoculation, in response to soil fertility improvement, through enhanced biological nitrogen fixation as compared to no inoculation (1.6 tonnes ha^{-1}). On the other hand, Hossain *et al.* (1996), could not demonstrate the effect of chickpea on soil organic C and total N, but reported an approximate doubling of the N mineralization potential of the soil.

2.4 Rhizobia Inoculation

Considerable work regarding the effect of rhizobia inoculation on yield of chickpea cultivars is available throughout the world, but very little work has been reported from Ethiopian studies. Inoculation is beneficial in two ways. First, it improves nodulation and dinitrogen fixation; second, it may increase a specific rhizobia population in the soil. Greater rhizobia population increases nodulation rates, thereby also increasing the N_2- fixation rate (Fomeg-As, 2004). Generally, legume yields are increased through N_2-fixation. The technology on legume inoculation or leguminous symbiotic/biological nitrogen fixation (BNF) is not yet widely disseminated to farmers in Ethiopia.

Each year, about 175 million tonnes of N is contributed by BNF globally, of which nearly 79 % is accounted for by terrestrial fixation. Therefore, symbiotic nitrogen fixation is of great importance not only in the production of leguminous crops but also in the global nitrogen cycle (Ben *et al*., 2008). The most important N_2 fixing agents in agricultural systems are the symbiotic associations between legumes and the rhizobia bacteria (Giller, 2001).

On average the estimated amounts of N fixed by chickpeas under regular precipitation 60 kg ha^{-1} (Unkovich and Pate, 2000; Abi-Ghanem *et al.,* 2012) and under drought stress conditions are 19–24 kg ha^{-1} (Carranca *et al*., 1999), respectively. There is increasing evidence that suggests that more N can be fixed by legume grain crops if they are inoculated more often or with more effective strains of rhizobia (Brockwell *et al*., 1989; Abi-Ghanem *et al.,* 2012).

2.4.1 Factors Affecting Nitrogen Fixation

The need to improve productivity of legumes as a global source of dietary protein has made it vital to understand the factors that influence nitrogen fixation (Schulze, 2004). Crop responses to inoculation with rhizobia inoculant are not as dramatic as those with inorganic N fertilizers. Being biological agents they are subjected to a range of hostile environments and their survival and efficiency is governed by several factors. Factors include soil factors, such as moisture (Griffith and Roughley, 1992; Issa and Wood, 1995), nutrients (Giller, 2001), temperature (Slattery *et al*., 2001), inoculant strain (Panda, 2011) and plant factors (Patterson and La Rue, 1983; Hardarson and Zapata, 1984; Sall and Sinclair, 1991; Abi-Ghanem, 2012).

2.4.1.1 Inoculant strains

Depending on the availability and effectiveness of the native rhizobia, N_2 fixation and productivity of chickpea can be increased in an economical feasible way by inoculating seeds with competitive strains of rhizobia (Ben *et al*., 2008). Despite being mentioned by some as a promiscuous host (Rivas *et al*., 2007), there is consensus that both nodulation and growth of chickpea can be improved by inoculation with effective strains (Giller, 2001).

Cleyet-Marel *et al*. (1990) described nodulation problems attributed to the rhizobial symbiont may be due to absence of appropriate strains, low population numbers, low infectiveness, poor survival in soil, or competition amongst strains of rhizobia. Legume inoculation is a way of assuring that the strain of rhizobium appropriate for the cultivar being planted is present at the proper time and in numbers sufficient to assure effective nodulation and nitrogen fixation.

Nodule initiation and development depends on the expression of host and microsymbiont genes. Variations in both host and bacterial genomes may affect the sequence of nodule development and the expression of the genes involved in nitrogenase activity and regulation. In some cases root hairs and nodules occur only where lateral roots emerge (Dongre *et. al.*, 1985).

Absence of nodules or nitrogen deficiency symptoms at flowering in unfertilized plants are indications of possible rhizobia absence or ineffectiveness. In this case, follow up in the form of an inoculum response trial using 'best' selected strains is a direct method to determine the role rhizobia play in the deficiency. Where available soil N is low (<l0 $\mu g\ g^{-1}$) and native rhizobia are absent or present in low numbers (<400 g^{-1} soil), inoculation with selected strains often boosts yields in excess of 50 percent (Singleton and Tavares, 1986; Rupela and Saxena, 1987).

2.4.1.2 Environmental factors

In crops grown on residual moisture, such as chickpea, the inoculated rhizobia cannot easily move downward with the growing root from the top soil where inoculation occurred, resulting in poor nodulation. In addition deep seeding results in a good crop stand but affects nodulation adversely (Panda, 2011).

In an experiment with strain IC2091 inoculated on five chickpea cultivars at four different soils in Syria, inoculation significantly increased nodule number for all cultivars analyzed in three testing sites but dry weight was significantly increased only in one of the locations, while grain yield increased (Cleyet-Marel *et al*., 1990).

Usually a higher mineral nitrogen content in the rhizosphere leads to poor N_2 fixation through inhibition of nodulation of chickpea (Namvar *et al*., 2011). On the other hand, small amounts of soil or fertilizer N often have a stimulatory effect on nodulation and N_2 fixation which is principally due to the positive effect of N on growth and plant establishment during the period between root emergence and the onset of active N_2 fixation (Giller and Cadisch, 1995).

Nitrogen fertilizer may not be required for chickpea production when growing conditions are favorable for N_2 fixation, although it may increase early vegetative growth and tissue N concentrations (Gan *et al*., 2008). Application of N fertilizer at 60 kg N ha^{-1} nearly doubled the seed yield of non-inoculated chickpea compared to the plants that did not receive N. When N_2-fixing *Rhizobium* was applied, both biomass and seed yield of chickpea were greatly enhanced regardless of N fertilizer (Gan *et al*., 2008). Increased seed yield due to N fertilizer or N_2-fixation was mainly due to increased number of pods and increased percentage of fertile pods (Kyei-Boahen *et al*., 2002; Gan *et al*., 2008).

Soil moisture deficiency has a pronounced effect on N_2 fixation because nodule initiation, growth, and activity are all more sensitive to water stress than carbon assimilation (Zahran and Sprent, 1986; Albrecht, 1994). The response of nodulation and N_2 fixation to water stress depends on the growth stage of the plants. Water stress imposed during vegetative growth was more detrimental to nodulation and nitrogen fixation than that imposed during the reproductive stage (Pena-Cabriales and Castellanos, 1993). Castellanos *et al.* (1996) indicated that N fixation of common bean (*Phaseolus vulgaris*) cultivar Bayocel was reduced from the control of 85 kg N ha^{-1} to 9 kg N ha^{-1} by moisture stress.

Unless well adapted, the size of rhizobial populations are likely to decline when exposed to harsh environmental conditions, particularly that of low soil moisture combined with high soil temperature (Rupela *et al*., 1987; Slattery *et al*., 2001). Temperature affects nodulation, survival and persistence of rhizobial strains in soil. High soil temperatures in tropical and subtropical

areas are a major problem for biological nitrogen fixation of legume crops (Michiels *et al*., 1994). Depending on their natural habitat, tolerance of rhizobia to temperature varies across various strains (Mohammadi *et al*., 2012). High root temperatures strongly affect bacterial infection and N_2 fixation in several legume species, including chickpea (Slattery *et al*., 2001), peanut (Kishinevsky, 1992) and bean (Hungria and Franco, 1993).

2.4.1.3 Plant factors

The efficiency of biological N fixation is also influenced by cultivar selection; significant differences were observed among pea cultivars for the percentage of plant N supplied by bacterial N fixation and also for the number of root nodules formed per plant in a greenhouse experiment (Abi-Ghanem, 2012).

Large variation in nodulation sensitivity to water deficit exists among soybean cultivars and the response of N_2 fixation rates to drought is related in part to nodule formation and growth (Serraj and Sinclair, 1998). Sall and Sinclair (1991) reported the presence of genetic variability in N_2 fixation sensitivity to drought among soybean cultivars. Genotypic variability was observed among these germplasm for ameliorating the effects of soil dehydration on nitrogen fixation rates. Hardarson and Zapata (1984) indicated that soybean cultivars were different in the extent to which they support N_2 fixation. This finding was also in agreement with Patterson and La Rue (1983), who found great variation in N_2 fixation between various maturity groups of soybean. Hardarson and Zapata (1984), using ^{15}N isotope methodology, reported great variability between eight soybean accessions in their ability to fix N_2 at different inorganic N levels; and significant differences were observed in percentage of N derived from the atmosphere (% Ndfa). Potential exists in breeding cultivars for improved traits associated with nitrogen fixation.

Chickpea genotypes varied significantly in their response to rhizobia inoculation for stover yield, nodule weight, and nodule number (Bhuiyan *et al*., 2008), but significant variability was observed for seed yield and nodulation in another experiment done on chickpea genotypes (Khanam *et al.*, 1994; Gupta and Namdeo, 1996).

Chickpea cultivars ‘ICCV-2’ and ‘Sarah’ were studied along with commercial multi-strain inoculants (Nitragin; LiphaTech,Inc., Milwaukee, WI), TAL 1148 (Nitragin 27A8; USDA 310),

TAL 480 (USAB 67) and a control to determine the effects of cultivar and inoculum on dry weight (DW) and N content of the legume, as well as soil mineral N, dry weight and N content of wheat in a continuous wheat-legume rotation. Chickpea pod dry weight as well as N contents of stem, shoot, and pods were significantly affected by cultivar. Year significantly affected DW and N content in all components, whereas the cultivar x year interaction had significant differences for leaf, shoot, and pod DW as well as leaf, stem, shoot, and pod N content. Neither inoculums, nor any of the inoculum interactions thereof, significantly changed DW or N content measurements (Bidlack *et al*., 2007).

2.5 Soil Fertility Improvement

In sub-Saharan Africa both yield and quality of crops are highly constrained by low N availability. Application of mineral fertilizer, addition of organic material and enhancing biological N_2 fixation are the main ways of improving the N availability to plants. Nitrogen is an essential nutrient for plant growth and development (Werner and Newton, 2005) due to its role in biochemical, physiological and morphological processes of plant production (Novoa and Loomis, 1981). Although this critically important element is abundant in the atmosphere, N is the most limiting element for crop growth worldwide.

The capacity of legumes to fix atmospheric N gives them an advantage over non-leguminous crops when grown on soils low in N. As such, they are an integral part of most small-landholder cropping systems (Bhatia *et al*., 2001).

2.5.1 N Benefit of Chickpea Rotation

Crop rotation is a systematic approach in which different crops are cultivated in a sequence that varies from year to year, as well as from season to season within a year. Chickpea is a beneficial rotation crop when it is used in sequence with cereals to assist in breaking weed and disease cycles, and also for providing soil nitrogen benefits. Nitrogen fixation by legumes has economic and environmental benefits. The economic benefits come from cost savings through reduced inputs of fertilizer N. The environmental benefits are primarily from reduced emissions of nitrous oxide, a potent greenhouse gas (Dixon and Khan, 2004).

Crops grown in previous years impact the amounts of residual soil water and nutrients available for subsequent plant growth. The best rotation sequence allows efficient use of available soil resources by the crop to increase yields at a system level (Gan *et al*., 2003). Legumes access atmospheric N_2 through BNF and so require minimal N fertilizer inputs. When part of this free N is made available to a subsequent crop, the use of legumes in a rotation can lead to a reduction in fertilizer N use and a reduction in input costs (van Kessel and Hartley, 2000; Herridge *et al*., 1998).

Inclusion of legumes increases soil fertility and consequently the productivity of succeeding cereal crops (Ghosh *et al*., 2007). Within this context, the growing of N_2-fixing grain legumes such as lupins (*Lupinus angustifolius* and *L. albus* L.), field pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), and faba bean (*Vicia faba* L.) within a cropping rotation would obviously offer an additional or alternative means of benefitting, if not fully restoring, soil N balance (Armstrong *et al*., 1997). Kumar and Prasad (1999) reported a saving of 25 kg N ha^{-1} in wheat when grown after a grain legume. The nitrogen economy was affected not only due to direct N addition through legume residues and its subsequent mineralization but also due to enrichment of soil with fixed N_2 from root exudates (Pawar and Jadhav, 1995).

The rotational benefits of chickpea, that is increased soil N (nitrate and total N) and cereal crop yields following chickpea appear to be more consistent. The contribution of five legumes to soil nitrogen and performance of succeeding maize (*Zea mays* L.) was studied in Kenya by Cheruiyot *et al*. (2001), and they reported that grain yield in maize succeeding legumes was 24-68 % higher than maize succeeding weed fallow. In the absence of N fertilizer input, maize (*Zea mays L*.) succeeding dolichos bean (*Lablab purpureus* L.) gave 20-40 % higher yield than maize after weed fallow treated with recommended 60 kg N ha^{-1} fertilizer rate. In the northern grains belt of Australia, Aslam *et al*. (2003) reported higher soil nitrate levels following chickpea than following wheat (average of +35 kg N ha^{-1}) resulting in higher yields of grain (+0.83 tonnes ha^{-1}, equivalent to 40 percent increase) and higher grain proteins (+14 percent). They concluded that the beneficial effects of chickpea were mediated through enhanced N supply to the wheat from the chickpea, rather than from disease break effects. Wheat following chickpeas out-yielded wheat after wheat by an average of 0.7 tonnes ha^{-1}. These studies demonstrate that the use of

grain legumes in rotation with cereals is a viable and preferable option to cereal-cereal sequences.

Although legumes are well known in improving soil N, some research findings indicated that only incremental changes in soil N associated with pulse crops were correlated to N_2 fixation, and they were highly variable. Contribution of BNF to N economy, when evaluated over the long term, depends on the level of N_2 fixation and the type of pulse. Walley *et al.* (2007) reported pulses such as faba bean, field pea, and lentils contributed positively to the overall N economy. In contrast, pulse crops that typically achieve only modest levels of N_2 fixation such as desi and kabuli chickpea, and common bean, are more likely to be either N neutral or contribute to a soil N deficit. Because of extreme variability in levels of N_2 fixation achieved, presumably reflecting variability in soil productivity as well as variations in local climate and weather, the N increment (positive input) of pulse crops is highly variable. Thus, the N contribution to a subsequent crop is difficult to predict with any certainty, particularly on a yearly or short-term basis.

2.6 Response of Chickpea to Moisture Stress

Drought is the main environmental constraint, which occurs in many parts of the world every year, often having devastating effects on crop productivity. Hence, improved tolerance to drought has been a goal in many crop improvement programs (Ludlow and Muchow, 1990). Drought tolerance is not a simple response, but is conditioned by many component responses, which interact and may be different for each crop, and the intensity and duration of water deficit. Moreover, most agronomical characters are expressed differently under normal and stress conditions and are known to be affected by environmental factors. Therefore, selection based on the phenotype would be difficult for such traits (Hittalmani *et al*., 2003).

Increasing yield is a major goal of plant breeders. Therefore, it is important to emphasize yield performance of chickpea cultivars under moisture-stress conditions. But variations in yield potential can arise from factors related to adaptation rather than to drought tolerance. Thus, drought indices based on yield loss under drought-conditions compared to normal conditions are being used in screening drought-tolerant genotypes (Mitra, 2001).

2.6.1 Terminal drought stress

Improved cultivars for arid regions must have drought resistance mechanisms to enable them to grow and survive in areas with low moisture availability (Zahran, 1999). Chickpea grown on stored soil moisture in subtropical areas is often exposed to drought during pod set and seed filling, the condition known as terminal drought.

Drought is the most important abiotic stress in chickpea worldwide. Terminal drought can reduce seed yields by 58–95 % compared with irrigated plants and reductions in pod production and grain filling are key factors impacting final seed yield (Leport *et al.,* 2006). Terminal drought decreases the rate of net photosynthesis of leaves during seed filling (Leport *et al.*, 1998, 1999; Davies *et al.,* 1999). Furthermore, N fixation also decreases during seed filling in chickpea (Hooda *et al.*, 1986; Kurdali, 1996), a response which is exacerbated by water deficit (Hooda *et al.*, 1989; Swaraj *et al.*, 1995). A high demand for assimilate from filling seeds when the supply of current assimilate is decreasing often results in an assimilate shortfall (Pate *et al.*, 1980; Egli and Crafts-Brandner, 1996). Consequently, alternative sources of assimilate are required to maintain seed filling and seed size, otherwise seeds are smaller or they take much longer to fill.

In assessing drought resistance of peanut genotypes, total biomass can be used to indicate their potential productivities under drought stress. Nageswara-Rao *et al*. (1994) reported that the productivities of drought resistant peanut lines under drought-stress conditions, as measured by total biomass, were higher than those of drought-sensitive genotypes. With differential responses to drought stress among peanut genotypes, high biomass production under drought stress of a resistant genotype could be due to its ability to produce high biomass under well-watered conditions, that is high potential, or its ability to maintain high biomass, or less reduction, under drought stress.

Zaman-Allah *et al*. (2011), reported variability among twenty chickpea genotypes based on the pattern of water extraction which clearly discriminated as tolerant and sensitive genotypes. Tolerant genotypes had a lower water uptake and a lower index of stomatal conductance at the vegetative stage than sensitive ones, while tolerant genotypes extracted more water than sensitive genotypes after flowering.

2.6.2 Drought and Nitrogen Fixation

In the rhizobia-legume symbiosis, the process of N_2 fixation is strongly related to the physiological state of the host plant. Therefore, a competitive and persistent rhizobial strain is not expected to express its full capacity for nitrogen fixation if limiting factors (e.g., salinity, unfavorable soil pH, nutrient deficiency, mineral toxicity, temperature extremes, insufficient or excessive soil moisture, inadequate photosynthesis, and plant diseases) impose limitations on the vigor of the host legume (Brockwell *et al*., 1995; Peoples *et al*., 1995a; Thies *et al*., 1995).

A favorable rhizosphere environment is vital to the legume-rhizobia interaction; however, both the magnitude of any stress effects and the rate of inhibition of the symbiosis usually depend on the phase of growth and development, as well as the severity of the stress. For example in a study made on peanut genotypes under water stress by Pimratch *et al.* (2008), biomass production and N_2 fixation decreased with increasing levels of drought stress. Genotypes did not significantly differ in reductions for biomass production, but did differ for reductions in N_2 fixation.

Symbiotic nitrogen fixation is highly sensitive to drought, which results in decreased N accumulation and yield of legume crops. The effects of drought stress on N_2 fixation usually have been perceived as a consequence of straightforward physiological responses acting on nitrogenase activity and involving exclusively one of three mechanisms: carbon shortage, oxygen limitation, or feedback regulation by nitrogen accumulation (Serraj *et al*., 1999a).

Soil moisture deficiency has a pronounced effect on N_2 fixation because nodule initiation, growth, and activity are all more sensitive to water stress than are general root and shoot metabolism (Zahran and Sprent, 1986; Albrecht *et al*., 1994). The response of nodulation and N_2 fixation to water stress depends on the growth stage of the plants. It was found that water stress imposed during vegetative growth was more detrimental to nodulation and nitrogen fixation than that imposed during the reproductive stage (Pena-Cabriales and Castellanos, 1993).

Water stress often has a negative effect on nodulation and seed yield in legumes but this effect can be reduced through N management. Gan *et al*. (2008) determined the synergistic effect of water stress and N fertilization on the morphology of nodules, biomass partitioning among shoot, roots and nodules, and seed yield in chickpea. The use of N fertilizer reduced the negative effect

of water stress by partitioning more biomass to roots. Stronger root systems allowed plants to absorb more water for the transport of fixed N (Gan *et al*., 2008).

2.6.3 Drought and Free Amino Acids

Drought stress can be a major limitation on yield of chickpea. Devi *et al*. (2009) observed a large range in the sensitivity of nitrogen fixation to soil drying among seventeen peanut genotypes. Loss in nitrogen fixation activity associated with soil drying might be limiting due to the need for high nitrogen amounts in both vegetative tissues and seeds of peanut. A positive correlation was found between the soil water content at which nitrogen fixation began decreasing and the amino acid concentration in the leaves of severely stressed plants.

The products of N_2 fixation, either amides (mainly asparagine) or ureides (allantoin and allantoic acid), are exported to the shoot via the xylem (Schubert *et al*., 1995; Walsh, 1995). Kirda *et al*. (1989) reported establishment and activity of the legume–rhizobia symbiosis to be extremely sensitive to drought stress. Consequently, legume productivity can be greatly depressed both by intermittent drought, which could occur at any time during the growing season when rainfall is inadequate, and by terminal drought, which occurs when stored soil moisture is depleted resulting in crop senescence (Saxena *et al*., 1993; Wery *et al*., 1994; Subbarao *et al*., 1995).

2.6.4 Measurement of N Fixation and N Products

Improvement of N fixation in stress is a strategy to increase yield back to that expected under well-watered conditions. For the best fixation in drought, a drought resistant genotype could be developed based on several physiological strategies: use of strain x host combinations that are stress tolerant; uncoupled or by-passed feedback inhibition; higher N fixation before stress so the shoot has high N concentrations to continue N metabolism during stress; a decrease in stress sensitivity by greater duration of N supply and metabolism during stress; and high N harvest index so the greatest portion of N in biomass is transferred to the yield portion. Soybean has been improved for stress tolerant N fixation in USA and Australia (Serraj *et al*., 1999b; Herridge and Rose, 2000) and common bean has also been improved in South America (Bliss *et al*., 1989; Henson *et al*., 1993).

There are different methods available for measuring N fixation, some methods that are least expensive are used for screening large numbers of genotypes (Hardason and Danso, 1993; Unkovich and Pate, 2000; Herridge and Rose, 2000). Amides and ureides fluctuations have been quantified as a means to understand N fixation limitations (Pate, 1973; Peoples *et al*., 1985 and 1987; Sinclair and Serraj, 1995). A rapid method for screening N fixation involves detection of a metabolite that is specific to fixation, not uptake, such as ureides in warm-season legumes. Xylem ureides (Herridge and Peoples, 1990) and shoot ureides (de Silva *et al*., 1996) are both effective and less expensive than other methods. Use of ^{15}N isotopes, as dilution or natural abundance, are the most precise but most expensive methods, and need not be used in routine screening except at selected stages to calibrate methods or check progress. Acetylene reduction, which indirectly measures N fixation at a point in time, is more difficult and best used on a small number of genotypes at the final stages of assessment (Sinclair *et al*., 2000), rather than large-scale screening.

In conventional N metabolism of major crops, nitrate can be reduced in the roots or the shoot. Ammonia from nitrate and nitrite reduction enters amino acid metabolism by glutamine synthetase and glutamate synthase (GS/GOGAT), then glutamate dehydrogenase, aspartate aminotransferase or asparagine synthetase (Buchanan *et al*., 2000). Typical transport forms of N to the shoot in the xylem include nitrate, certain amino acids such as aspartic acid (ASP), glutamic acid (GLU) and frequently the amide amino acids asparagine (ASN) and glutamine (GLN). Evidence for transport forms of N are reported for crops including wheat, broccoli, faba bean, chickpea, cowpea, pea, sunflower and maize (Pate, 1973; Simpson *et al*., 1982; Peoples *et al*., 1985 and 1987; Shelp, 1987). Legumes that fix nitrogen transport N metabolites in the form of amino acids and amides (mainly ASP, GLU, ASN, GLN), ASN being in the greatest amount (Meeks *et al*, 1978; Sieciechowicz *et al*., 1988). This is the case for cool season legumes such as pea, lentil and lupin. Warm-season legumes, for example, cowpea, pigeon pea, soybean, and to a small extent faba bean and chickpea, produce and transport N from fixation as ureides (Streeter, 1991; Sinclair and Serraj, 1995).

Nitrogen fixation is also controlled by a feedback regulation of N metabolites (Lukaszewski *et al*., 1992; Serraj *et al*., 1999b). In soybean, a ureide producer, the rate controlling step in ureide metabolism in the shoot, is the enzyme allantoate amidohydrolase (Lukaszewski *et al*., 1992).

Ureides accumulate under stress in leaf petioles and shoot material, and shoot metabolite, possibly asparagine, appears to be transported in the phloem to the nodules as a feedback inhibition signal if N metabolites are building up in the leaf. Ureide concentration, specific to N fixation, was used to indicate the amount of fixation. A high shoot ureide concentration was used to indicate drought sensitivity (de Silva *et al.*, 1996). The screening methodology was to phenotype based on lower feedback inhibition in the shoot of soybean (Serraj *et al.*, 1999b).

3. Response of Chickpea Cultivars to Seeding Dates across Agro-ecological sites of Southern Ethiopia

3.1 Introduction

Chickpea is an integral part of cropping systems in southern Ethiopia where it is rotated with cereals as part of soil fertility maintenance and as a source of cash. Chickpea is normally grown using residual moisture. The seeding normally starts at the end of August after maize (*Zea mays*), teff (*Eragrostis teff*) or wheat (*Triticum aestivum*) are harvested.

Yield of chickpea is influenced by several factors including genotype, growing season, geographical site, and agronomic practices (Tawaha *et al*., 2005). In Ethiopia low productivity (1.7 tonnes ha^{-1}) of chickpea is due to use of local landraces with low yield potential and poor agronomic practices such as delayed seeding date caused by late harvest time of the preceding crop, broadcasting seed, use of a furrow turning plough for covering the seed, and variable seeding depth.

Early maturing high-yielding (2.5 – 3.0 tonnes ha^{-1}) kabuli cultivars with market-preferred traits such as large seed and desirable seed color, have not reached farmers on a large scale and hence the productivity of the crop is low. The improved cultivars have high yield potential, up to two or three folds greater than the local landrace (Asfaw *et al*., 2010). In addition, the released cultivars have better stress tolerance, wider environmental adaptability, and better food quality characteristics than the local landrace (Dadi *et al*., 2005).

In chickpea growing areas of southern Ethiopia terminal drought stress is a major abiotic stress affecting chickpea productivity resulting from lack of rainfall during flowering, podding and seed filling stage. Similar problems in chickpea were reported in Iran (Sabaghpour, 2004). The problem is more serious when using late maturing cereal crop cultivars combined with the use of chickpea cultivars that require a longer period to mature.

Introduction of early maturing, high yielding, and market-preferred chickpea cultivars will increase chickpea productivity in the area and chickpea production at the national level also capture a market premium. In addition, studying the agronomy of these improved cultivars such

as appropriate seeding date and inoculation, will contribute to efficient utilization of available moisture and to yield increase.

The best chickpea seeding date following a cereal crop has not been studied in southern Ethiopia to date. The development and release of new chickpea cultivars with high yield potential (largely due to greater regional adaptation, improved disease and drought tolerance) is one component of the agronomic package required to increase productivity of chickpea in southern Ethiopia. Therefore, this experiment was planned to study the effect of seeding date on yield and yield attributes of improved chickpea cultivars across agro-ecologies of southern Ethiopia.

3.2 Materials and Methods

3.2.1 Description of the Study Sites

The experiment were conducted in 2011 and 2012 at three sites in southern Ethiopia, namely Butajira, Wolaita, and Halaba (Table 3-1). The crop history in the experimental area indicated that in 2011 in Wolaita and Butajira maize (*Zea mays*) was the main-season (May-August) crop, teff (*Eragrostis teff*) in Butajira and potato (*Solanum tuberosum*) in Wolaita in 2012 main-season. In Halaba the field was occupied by chat (*Khata edulis*) for the last 7-8 years until 2011, which is a perennial tree crop grown for the stimulant effect of the leaf, and teff (*Eragrostis teff*) in 2012.

Table 3-1 Geographical location, climatic characters and soil texture of the research sites

Sites/ Locations	Soil type	Annual Rainfall (mm)	Altitude (m ASL)	Mean Annual Temp.	Position
Butajira	Vertisol	1100	1921	18°C	Latitude : 08° 12` 29.9`` N Longitude : 38° 27` 42.1`` E
Wolaita	Clay loam	1150	1906	20°C	Latitude : 07° 01` 03.1`` N Longitude : 37° 54` 17.0`` E
Halaba	Sandy loam	800	1800	21°C	Latitude : 07° 20` 47.9`` N Longitude : 38° 06` 29.6`` E

Source: IDRC project baseline survey report, 2010

3.2.2 Weather and Soil Condition of the Study Sites

Both Wolaita and Butajira sites typically have more rainfall than the Halaba site. The areas are characterized by bimodal rainfall distribution pattern with major rainfall from March to September, with July and August having the highest rainfall (Table 3-2). The soil in Halaba is sandy in texture (Table 3-1), and was accompanied with warm temperatures which resulted in moisture stress in the area.

Table 3-2 Monthly rainfall (mm) and mean temperature (°C) distribution of the research locations in 2011 and 2012 cropping seasons

Month	2011						2012					
	Halaba		Wolaita		Butajira		Halaba		Wolaita		Butajira	
	RF(mm)	T(°C)	RF(mm)	T(°C)	RF(mm)	T(°C)	RF(mm)	T (°C)	RF(mm)	T (°C)	RF(mm)	T (°C)
January	9	22	13	20	0	19	0	22	0	20	0	17
February	21	23	20	21	0	20	0	22	0	23	0	18
March	37	23	43	22	51	20	36	23	45	22	20	19
April	48	23	63	22	49	22	48	22	181	19	111	19
May	105	22	372	20	49	21	5	23	72	20	67	19
June	121	20	171	18	149	20	42	21	172	18	111	18
July	138	20	158	18	218	19	161	20	197	17	221	17
August	113	20	173	18	180	18	85	20	170	18	95	16
September	49	21	61	19	86	19	63	21	135	18	100	17
October	0	21	18	20	0	18	4	21	13	20	5	18
November	7	22	112	19	14	18	7	22	33	20	21	19
December	0	21	3	19	0	16	0	21	0	20	0	17

RF=Rainfall, T= Temperature

3.2.3 Cultivars and Seeding Dates

The field experiment included five chickpea cultivars (Table 3-3) and three seeding dates. At Halaba, these were September 2, September 13 and September 22 in 2011; September 3, September 14, September 23 in 2012. At Wolaita, September 7, September 18 and September 27 in 2011; September 4, September 15, and September 24 in 2012. At Butajira, September 5, September 16 and September 27 in 2012. The experimental unit was replicated three times at each location and year. The experiment at each location was arranged in a randomized complete block design where five cultivars and three seeding dates were arranged in a factorial combination. All chickpea cultivars were inoculated with commercial *Rhizobium* (Becker Underwood-Nodulator) inoculant imported from Canada. This peat based commercial inoculant was used because there is no registered inoculant for chickpea in Ethiopia. Recommended di-ammonium phosphate (DAP) fertilizer at the rate of 60 kg ha^{-1} was broadcasted on each block.

Table 3-3 Names and pedigree code of chickpea cultivars included in field experiment and their agronomic features

Cultivars	Pedigree code	Type	Origin	Growth Habit	Seed coat color	100-seed wt. (g)	Year of release in Ethiopia
Worku	ICCL 820104	Desi	ICRISAT	Semi erect	Golden	33	1994
Natoli	ICCX-910112-6	Desi	ICRISAT	Semi erect	Golden brown	32	2007
Habru	FLIP-88-42c	Kabuli	ICARDA	Semi erect	White	37	2004
Ejere	FLIP-97-263c	Kabuli	ICARDA	Semi erect	Creamy white	41	2005
Ethiopian landrace	n.a	Desi	Ethiopia	Spreading	Golden	12	n.a

The plot size was 2 m x 4 m with 0.1 m between plants within a row and 0.3 m between rows and a total of 6 rows per plot. Weed control was done manually twice during the growing season. Data were recorded from the middle four rows (1.2 m x 4 m) and used for the analysis. The experiment was conducted for two consecutive years in 2011 and 2012.

3.2.4 Measurements of Agronomic and Physiological Parameters

Days to emergence was recorded for each plot when more than 50 % of the plants emerged. Similarly, days to flowering and maturity were recorded when more than 50 % of the plants in each plot attained flowering and 90 % reached physiological maturity based on yellowish pod color change. At the time of maturity, five plants were randomly selected and tagged from the four middle rows and their height from the ground to the tip was measured using a ruler. From the five randomly selected sample plants at maturity, the number of pod bearing branches (both primary and secondary) were counted and averaged to give number of branches plant^{-1}.

The same five plants were also used to measure parameters including number of pods plant^{-1}, number of seeds pod^{-1}, and hundred seed weight for each plot. All the plants from the middle four rows were manually harvested and brought together in a plastic sheet for each plot. The harvested samples were sun-dried and threshed manually for each plot. The grain was put in a cloth sack and weighed to give yield per plot which was later converted into tonnes ha^{-1}. On the same date, seeds were sampled and taken to the laboratory to measure their moisture content using a seed moisture meter (Model HOH- Express He 50, Germany). The final grain yield of each plot was adjusted to 12 percent seed moisture content.

Grain samples were taken from each treatment and ground using a cyclone mill to analyze nutrient concentrations. An acid digest of ground chickpea grain was conducted according to the method of Thomas *et al*. (1967). Between 250-300 mg of finely ground grain samples were weighed into glass digestion tubes and 5 ml of concentrated sulfuric acid (H_2SO_4) was added. Samples were placed on digestion block at 360°C for 30 minutes. Following this, samples were removed from the digestion block, allowed to cool, and 0.5 ml H_2O_2 was added. Samples were then placed on the digestion block an additional three times for 30 minutes, adding H_2O_2 after each heating period. Finally samples were placed on the digestion block for 1 hour. After samples were allowed to cool, distilled water was added to dilute the final volume of the sample

to 75 ml to achieve a final concentration within the detection limit of the instrumentation. Then analysis for grain total nitrogen and phosphorus was done using an Auto-analyzer (Technicon™ auto-analyzer) colorimetry. The N value was multiplied by 6.25 to calculate the protein concentration.

3.2.5 Statistical Analysis

Data analysis was done using the PROC MIXED procedure of 9.3 SAS software (Little *et al*., 1996). Single environment and multi-environment data were subjected to analysis of variance as a mixed model. For single environment analysis, the effect of replication was considered random whereas the effects of cultivar and seeding dates were considered as fixed. In multi-environment analyses, the effects of cultivar, seeding date and cultivar x seeding date were fixed, and other variables were random. Least squared means (LSmeans) were computed for fixed effects using the LSMEANS statement. Standard errors of LSmeans were estimated and pair-wise tests of significant differences were performed using the PDIFF statement (at $P<0.05$ for significance).

3.3 Results

3.3.1 Weather Conditions during Cropping Seasons

At the pod filling stage and towards maturity there was an increase in moisture as the areas received some rain in November, 2011. This supported late flowers for pod setting mainly for late maturing cultivar.

During the cropping season of the experiment, the three sites received some rainfall in September but minimal rainfall in October in 2011 and 2012. The decline in soil moisture was greater in Halaba 2011 than in Wolaita and Butajira in 2011. This was due to Sandy loam soil texture and high temperature (20-23 $^{\circ}$C) in the area (Table 3-1 and 3-2). The impact of low moisture was observed on plant vigor and other morphological traits (Chapter 6).

3.3.2 Combined Analysis Across Years and Locations.

Data from five environments, two locations in 2011 and three locations in 2012, were used in a combined analysis. Due to damage occurred on 60% of the plots in Butajira experiment, the 2011 trial was dropped and result of five environments were included for combined analysis. Analysis of variance (Table 3-4) indicated locations over years (site year) was non-significant for all parameters but site year x cultivar for characteristics like branch number, 100-seed weight, and grain yield, and site year x cultivar x seeding date for flowering, maturity, and pod number were significant ($p<0.05$), indicating that the performance of cultivars for the specified characteristics was different across locations and over years so separate analyses were conducted for these parameters. The cultivar effect under combined analysis was highly significant for plant height ($p<0.01$).

Table 3-4 Analysis of variance (p-values, mean and standard error) for yield, flowering, yield components, and yield quality characteristics across five environments (SY) in seeding date trials in Ethiopia for five chickpea cultivars

Effects	Days to Flower	Days to Matu-rity	Plant Height (cm)	Branch No	Pod No	100 Seed Weight	Yield t ha^{-1}	Seed Protein %	P mg g^{-1}
Cultivar (V)	0.001	0.004	0.002	0.011	0.032	0.001	0.001	0.19	0.35
Seeding (S)	0.69	0.94	0.89	0.86	0.45	0.65	0.015	0.78	0.48
V * S	0.38	0.74	0.12	0.76	0.57	0.81	0.85	0.97	0.88
Site Year(S Y)	0.09	0.08	0.13	0.08	0.96	0.11	0.08	0.09	0.08
S Y * V	0.13	0.15	0.33	0.049	0.022	0.007	0.011	-	-
S Y * S	0.047	0.05	0.06	0.23	0.11	-	0.06	0.33	0.16
S Y * V * S	0.002	0.004	-	-	0.022	0.29	-	0.002	0.003
Mean	44.4	95.6	37.5	11.2	43.2	24.8	1.51	18.3	2.7
SE	0.53	0.86	0.37	0.37	1.45	0.51	0.06	0.13	0.06

Probability of significant: $p<0.05$

Combined analysis revealed that grain yield varied across environments therefore, results from different locations and year are presented separately. Cultivar Natoli produced better grain yield of 1.04 and 1.41tonnes ha^{-1} in Halaba and Wolaita respectively, in 2011 (Fig. 3-1A). In Halaba 2012 all improved cultivars were similar but the Ethiopian landrace had the lowest yield (0.71 tonnes ha^{-1}). In Wolaita, Natoli recorded the maximum yield 1.99 tonnes ha^{-1}, and in Butajira Natoli (3.4 tonnes ha^{-1}) and Habru (3.6 tonnes ha^{-1}) were the best yielding cultivars (Fig. 3-1 B). Yield from the early seeding date (1.85 tonnes ha^{-1}) and from the mid-seeding date (1.83 tonnes ha^{-1}) gave 8 % and 6 % yield advantage, respectively, over late seeding (1.71 tonnes ha^{-1}).

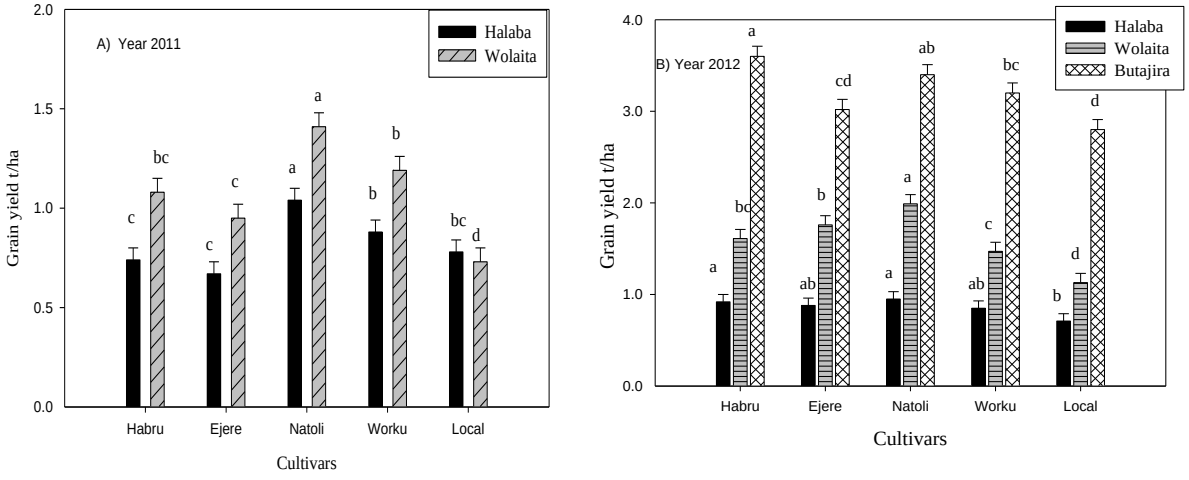

Figure 3-1 Mean grain yield (tonnes ha^{-1}) of five chickpea cultivars across different seeding dates in Halaba and Wolaita 2011 (A), and Halaba, Butajira and Wolaita (B) in 2012 trials. Bar graphs with the same letter in the same year at each location are not significantly different at $p<0.05$.

Combined analysis indicated 100-seed weight of cultivars varied across environments; therefore, results are presented separately for each year. The 100-seed weight was higher for the two improved kabuli cultivars (Fig. 3-2 and 3-3) compared to the rest. Habru (33.6 g) and Ejere (35 g) had higher 100-seed weight than the rest of cultivars at Halaba in 2011(Fig. 3-2), whereas in the same year at Wolaita, Ejere (35.8 g) had greater 100-seed weight followed by Habru (31.1 g). In 2012 the two cultivars, Habru and Ejere, had the highest 100-seed weight in all the locations

(Fig. 3-3). The Ethiopian landrace gave the lowest 100-seed weight in all locations in two seasons (Fig. 3-2 and 3-3).

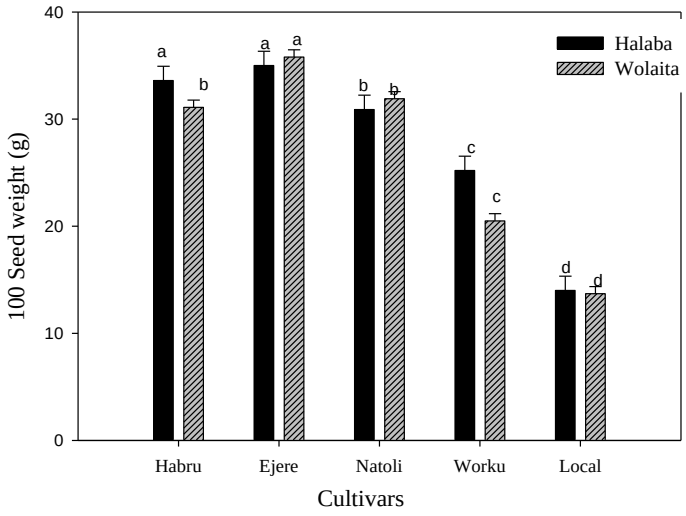

Figure 3-2 Mean performance of 100-seed weight of five chickpea cultivars in Halaba and Wolaita 2011 seeding date trials

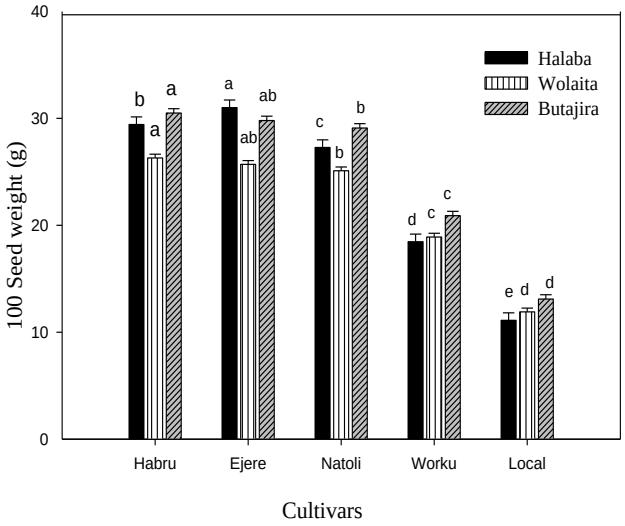

Figure 3-3 Mean performance of 100-seed weight of five chickpea cultivars in Halaba, Butajira and Wolaita 2012 seeding date trials

Interaction effect of cultivar by seeding date at each location over two growing seasons was significant for flowering, maturity and pod number (Table 3-5 and 3-6). Flowering and maturity of cultivar Natoli tended to be late in all locations in different years when seeded early, except in Wolaita 2012 where Natoli ranked third at mid-seeding date. On the other hand, Habru and Ejere, when seeded at mid and late dates, tended to flower and mature early in all locations. In Wolaita 2012, Ejere was late, followed by Natoli (Table 3-5) when seeded early. Interaction effects on pod numbers in all locations and years indicated that Worku and the Ethiopian landrace had the best performance at various combinations of seeding date. Worku at early and mid-seeding dates, and the Ethiopian landrace at the mid-seeding date, produced more pods compared to other treatment combinations (Table 3-6).

Table 3-5 Interaction effects of cultivar and seeding dates on days to flowering and maturity of five chickpea cultivars at three different locations in 2011 and 2012 trials

Cultivar * Seeding Date	Flowering					Maturity				
	2011		2012			2011		2012		
	Halaba	Wolaita	Halaba	Wolaita	Butajira	Halaba	Wolaita	Halaba	Wolaita	Butajira
Habru- 1st Seeding	37.0^{cde}	29.3^{gh}	44.0^{de}	52.0	44.0	89.0^{b}	113	82.3^{cde}	102^{bc}	86.0
Habru- 2nd Seeding	33.3^{f}	31.3^{g}	42.0^{ef}	47.3	42.3	89.3^{b}	116	84.0^{abc}	92.6^{ef}	89.0
Habru-3rd Seeding	33.6^{f}	37.6^{ef}	46.6^{bcd}	46.3	51.6	83.3^{cd}	116	82.3^{cde}	91.3^{f}	96.3
Ejere- 1st Seeding	37.3^{cde}	24.3^{i}	45.0^{cde}	47.0	43.0	83.6^{cd}	114	82.3^{cde}	108^{a}	84.6
Ejere- 2nd Seeding	34.6^{ef}	28.0^{h}	41.6^{ef}	46.6	42.3	82.3^{d}	117	85.0^{abc}	100^{bc}	89.6
Ejere-3rd Seeding	35.0^{def}	40.3^{cd}	39.6^{f}	44.0	50.0	82.6^{d}	117	79.6^{de}	83.0^{g}	93.3
Natoli- 1st Seeding	41.6^{ab}	41.3^{bcd}	58.3^{a}	64.6	53.0	94.6^{a}	125	86.6^{ab}	104^{ab}	92.6
Natoli- 2nd Seeding	44.3^{a}	43.3^{ab}	50.3^{b}	59.6	54.0	91.0^{b}	127	87.6^{a}	99.6^{cd}	95.3
Natoli-3rd Seeding	43.0^{a}	41.3^{bcd}	50.0^{b}	60.3	58.3	89.6^{b}	127	84.6^{abc}	102^{bc}	103
Worku- 1st Seeding	39.0^{bc}	40.0^{cd}	48.6^{bc}	50.0	45.3	89.3^{b}	115	84.3^{abc}	100^{bc}	90.6
Worku- 2nd Seeding	43.3^{a}	44.6^{a}	48.6^{bc}	55.6	44.0	90.3^{b}	116	85.3^{abc}	98.6^{cd}	93.6
Worku-3rd Seeding	37.6^{cd}	42.0^{bc}	48.6^{bc}	49.3	54.6	85.6^{c}	116	83.3^{bcd}	96.0^{de}	97.0
Local- 1st Seeding	37.3^{cde}	37.0^{f}	49.3^{b}	47.6	45.0	85.6^{c}	112	82.3^{cde}	99.0^{cd}	85.0
Local- 2nd Seeding	36.6^{cde}	43.3^{ab}	45.0^{cde}	54.0	39.6	84.6^{cd}	112	75.3^{f}	102^{bc}	90.3
Local-3rd Seeding	36.0^{def}	39.3^{de}	48.0^{bc}	48.0	52.6	84.3^{cd}	112	78.6^{ef}	101^{bc}	93.0
SE	1.15	0.72	1.37	2.1	1.02	0.89	1.23	1.45	1.43	1.23
LSD	2.99	2.09	3.97	6.1	2.9	2.55	3.6	4.18	4.07	2.88
p- Value	0.01	0.001	0.011	0.138	0.138	0.01	0.945	0.001	0.001	0.145

Means within a column and treatment followed by the same letters are not significantly different ($p < 0.05$).

Table 3-6 Interaction effects of cultivar and seeding dates on pod number of five chickpea cultivars at three different locations in 2011 and 2012 trials

Cultivar * Seeding Date	Pod Number				
	2011		2012		
	Halaba	Wolaita	Halaba	Wolaita	Butajira
Habru- 1st Seeding	52.8[a]	34.1	16.4	31.0	71.6
Habru- 2nd Seeding	36.8[bcde]	52.8	19.1	31.6	62.3
Habru-3rd Seeding	32.1[bcde]	33.3	21.6	30.0	67.2
Ejere- 1st Seeding	23.8[e]	27.6	15.7	32.2	70.6
Ejere- 2nd Seeding	31.0[cde]	44.8	17.7	31.5	72.3
Ejere-3rd Seeding	43.4[abc]	35.7	17.1	30.2	69.2
Natoli- 1st Seeding	31.3[cde]	72.5	19.5	30.6	55.3
Natoli- 2nd Seeding	38.0[bcde]	51.4	18.9	32.1	50.0
Natoli-3rd Seeding	24.8[e]	37.6	20.8	28.5	48.5
Worku- 1st Seeding	54.0[a]	78.1	19.3	40.3	68.1
Worku- 2nd Seeding	42.1[abcd]	93.4	20.6	40.0	72.3
Worku-3rd Seeding	54.7[a]	43.8	18.6	42.5	62.9
Local- 1st Seeding	28.3[de]	88.2	20.1	40.3	74.1
Local- 2nd Seeding	46.0[ab]	87.9	24.8	41.5	71.1
Local-3rd Seeding	52.7[a]	62.5	18.5	42.2	75.6
SE	6.09	9.4	2.6	4.3	4.67
LSD	14.36	25	7.3	11.8	13.36
p- Value	0.001	0.072	0.767	0.998	0.785

Means within a column and treatment followed by the same letters are not significantly different ($p < 0.05$).

3.4 Discussion and Conclusion

The best chickpea seeding date following a cereal crop has not been studied in southern Ethiopia to date. Farmers traditionally seed chickpea on residual moisture after the main-season crop is harvested. The development and release of new chickpea cultivars with high yield potential (largely due to greater regional adaptation, improved disease and drought tolerance) is one component of the agronomic package required to increase productivity of chickpea in southern Ethiopia. Farmers traditionally use local landraces and seeding date usually depends on the harvest time of the preceding crop, which in most cases has delayed chickpea seeding time. As a result of the delayed seeding time, chickpea is frequently exposed to terminal drought. Therefore, this experiment was planned to study the effect of seeding date on yield and yield attributes of improved chickpea cultivars across agro-ecologies.

The early and mid-seeding date increased grain yield (1.84 tonnes ha^{-1}) by 7 percent over late seeding (1.71 tonnes ha^{-1}). Kabir *et al*. (2009) observed a yield reduction due to late seeding time. Results obtained in this experiment were in agreement with other research conducted in Jordan and in India (Dixit *et al.*, 1993; Al-Rifaee *et al.*, 2007; Prasad *et al*., 2012) that found reduced yield with late seeding. Tiwari and Meena (2014) conducted an experiment in India with three seeding dates, November 10, 25 and December 5, and reported mid-seeding date to give yield (1.5 tonnes ha^{-1}) increases of 12 and 32 percent more than early (1.3 tonnes ha^{-1}) and late seeding (1.1 tonnes ha^{-1}) dates, respectively. Ray *et al*. (2011) in West Bengal with a first seeding on 20 November and a second seeding on 6 December, found that early seeding increased seed yield (1.5 tonnes ha^{-1}), number of pods $plant^{-1}$, seed pod^{-1} and test weight compared to late seeding (1.2 tonnes ha^{-1}).

The effect of seeding date on grain yield was partly through the increase of 100-seed weight and branch number. Early seeding would be key to optimized water use, increased biomass, and hence, more assimilate to the grain. This was observed from cultivars such as Natoli, Habru and Ejere, that all had more branches and higher 100-seed weight than the local landrace. In contrast, the local landrace had more pods per plant and a greater seed number compared to other cultivars at all seeding dates although grain yield was inferior due to reduced 100-seed weight (Fig. 3-2 and 3-3).

This experiment has also shown that grain yield and 100-seed weight variation was also dependent on cultivar and agro-ecological sites.

Analysis of grain yield demonstrated significant differences among cultivars and the three seeding dates, but there was no interaction between seeding date and cultivar. Cultivar Natoli produced high grain yield of 1.04 and 1.41tonnes ha^{-1} in Halaba and Wolaita, respectively, in 2011 (Fig. 3-1A). In Halaba 2012 all improved cultivars performed similarly but the Ethiopian landrace had much lower yield (0.71 tonnes ha^{-1}). In Wolaita, Natoli produced the highest yield of 1.99 tonnes ha^{-1}, and in Butajira Natoli (3.4 tonnes ha^{-1}) and Habru (3.6 tonnes ha^{-1}) were the best yielding cultivars (Fig. 3-1 B). Similar to this study other researchers found yield variation dependent on cultivar and environment (Valimohammedi *et al*., 2007; Shamsi, 2009; Kabir *et al.*, 2009; Tiwari and Meena, 2014). This higher grain yield of improved cultivars was mainly due to higher 100-seed weight. This was evident from lower yield obtained from the local landrace despite having more pods and seed number per plant but an inferior 100-seed weight. Higher yield of improved cultivars can also be associated with early seeding date that provides the congenial environmental conditions for the growth and development of the plant and hence more vigorous growth which resulted into higher yield attributes. Previous findings also support these results (Kumar *et al*., 2008; Kabir *et al*., 2009; Prasad *et al.,* 2012).

The 100-seed weight was not influenced by the variation in seeding dates; this finding is in conformity with those of Nawaz *et al.* (1995); Kumar *et al*. (2008) and Kabir *et al*. (2009). The 100-seed weight was higher for Ejere (35 g) and Habru (33.6 g) cultivars (Fig. 3-2 and 3-3) compared to the rest. The 100-seed weight is an inherent genetic characteristic which is not usually affected by environmental changes unless the change is extreme. The variation in seed weight observed was only from cultivar differences but not environmental effects.

The interaction effect of cultivar by seeding date showed significant difference in case of flowering, maturity and pod number $plant^{-1}$ (Table 3-5 and 3-6). A similar interaction effect was reported by Kabir *et al*. (2009) for pod number $plant^{-1}$, flowering and maturity but Prasad *et al*. (2012) found an interaction effect for flowering and maturity. In agreement with other studies (Photiades, 1984; Husnain *et al*., 2015) flowering and maturity of the cultivar Natoli tended to be late (Table 3-5) when seeded early and at mid-seeding date. This might be due to favorable

temperature during crop growth period resulting in increased number of branches and vigorous growth, which may be responsible for extended flowering and maturity time. In contrast, Habru and Ejere seeded at mid and late seeding dates, tended to flower and mature early in all locations. This is because of a short growing period, and later seeded chickpeas are able to compensate through shortening their vegetative phase and flowering at temperatures more conducive to subsequent pod development. Any photoperiod requirement was satisfied and growth phase variation was driven by temperature. This result was in agreement with Jenkins and Brill (2011); and Khajehpour (2000), where they reported that late seeding of chickpea resulted in early maturity by shortening the vegetative stage before flowering. Contrary to this, other reports indicated delayed flowering and maturity of chickpeas due to late seeding (Tiwari and Meena, 2014; Prasad *et al.*, 2012). This was associated with decreased temperature due to delayed seeding date.

The highest number of pods was obtained from early and mid-seeding dates. Cultivar Worku and Ethiopian landrace had the best performance at both early and mid-seeding dates (Table 3-6). This result was also reported earlier by Tiwari and Meena (2014), Kabir *et al*. (2009), and Dixit *et al.* (1993). This might be due to favorable temperature and moisture during crop growth period resulting in increased branch number, more number of pods plant^{-1}, and better source sink relationship.

Early flowering and maturity, larger seed size, and desirable seed color of the two kabuli chickpea cultivars Habru and Ejere could be considered an economic advantage and would be recommended for cropping systems in Halaba and Wolaita areas. Halaba is a moisture stress area so early maturing cultivars may escape terminal drought stress. Late maturing cultivars like Natoli had more pod number at early and mid-seeding date than late seeding in Halaba and Wolaita areas. Since Natoli was late for flowering and maturity early seeding would be advisable for maximum yield advantage. In addition early maturing cultivars were the best fit for a double cropping system that may contribute to food security in the study area.

The research revealed that seeding date significantly affected grain yield. Interaction of cultivars and seeding dates was significant and varied across years and locations for days to flowering, days to maturity and pod number plant^{-1}. Therefore, we accept the hypothesis and concluded that total yield and grain yield increases were resulted from early seeding. The cultivation of cereals

and legumes in nutrient-deficient soils, coupled with inadequate or no crop management (fertilizer, proper seeding time) resulted in a low crop yield. High yield, early maturity and disease resistance are essential traits that need to include in improved cultivars. They also need to be combined with traits that reduce input cost such as increased N_2 fixation capacity.

4. Preface

The seeding date experiment (Chapter 3) addressed one of the major agronomic problems of chickpea in southern Ethiopia which has not been studied to date. The nationally released chickpea cultivars with high yield potential, wide adaptation, improved disease resistance and drought tolerance were mainly distributed and produced in central and north-western part of Ethiopia. In southern Ethiopia farmers have used local landraces and the dependence of chickpea seeding date on the harvest time of the preceding crop has been identified as one factor for the delay of chickpea seeding date resulting in the exposure of the crop to terminal drought.

This research revealed the best cultivars that fit for the cropping system in the study areas. Early flowering and maturity, larger seed size, and desirable seed color of the two kabuli chickpea cultivars Habru and Ejere were recommended in order to increase the chickpea productivity. Due to the high population density that exists in southern Ethiopia, frequent cropping of arable land is a common practice. In addition farmers in the area cannot afford the cost of fertilizers for cereal crops grown in the main-season. As a result, cultivation of cereals and legumes in nutrient-deficient soils, coupled with inadequate crop management (fertilizer, proper seeding time) result in low crop yield. Therefore, to address the low yield of chickpea due to nutrient deficiency and to maximize the economic benefit of chickpea, the research to evaluate the response of chickpea cultivars to *Rhizobium* inoculation was conducted. This experiment was intended to identify the best cultivars and *Rhizobium* combination that would result in greatest N_2 fixation and high grain yield.

4. Response of Chickpea Cultivars to rhizobia Inoculation across Agro-ecological sites of Southern Ethiopia

4.1 Introduction

In Ethiopia pulses are grown throughout the country. During the 2012-13 cropping season, chickpeas accounted for 14 percent of cropped land area (Central Statistics Agency of Ethiopia and World Bank, 2013). Chickpea production is concentrated in the north, north-western and central regions, which together accounted for 92 % of national production (International Food Policy Research Institute, 2010). Currently, only a very small area is used for chickpea production in the southern part of Ethiopia. This could be associated with minimum resource availability, such as suitable land, and improved cultivars, lack of appropriate technology, or extension activities dedicated to chickpea production in the region. Considering the economic, nutritional, and agronomic benefits of chickpea, the southern part of Ethiopia has the potential for expansion of chickpea production; however, research is needed to develop basic crop management for successful production.

Analysis of cost of production and market opportunities of desi and kabuli chickpeas in Ethiopia demonstrated that farmers using improved management would gain higher return by switching from the production of traditional desi chickpea to the production of high yielding kabuli cultivars (Shiferaw *et al*., 2007). Nitrogen is known as one of the most limiting factors for crop production in the study areas, which is required in large quantities. Most lands are nitrogen deficient and the deficiency is aggravated mainly due to continuous planting of non-legume crops without application of fertilizer.

One of the improved management practices is inoculation of chickpea for nitrogen fixation. Pulses have nitrogen fixing properties that can reduce N fertilizer usage for its own growing need and for cereals in the following season by up to 60 % (International Food Policy Research Institute, 2010). The fact that cereal production causes higher soil nutrient depletion, rotating between pulses and cereals will not only contribute towards maintaining soil health but can also reduce the cost of production for the resource-poor farmers as well as the country's fertilizer usage. Those soils with continuous cereal cultivation are generally deficient in *Rhizobium* species which are required for successful nitrogen fixation in legume crops. There is a potential

to increase chickpea productivity in the region through the application of an ideal strain of nitrogen fixing bacteria either to the seed or to the soil.

The inclusion of legumes and rhizobia in a cropping system does not always guarantee the attainment of optimal level of symbiotic nitrogen fixation in the field. Several environmental factors including drought, temperature, and soil status are known to negatively affect the symbiosis and nitrogen fixation process and, thus, reduce the actual amount of nitrogen fixed by a given legume in the field (Serraj and Adu-Gyamfi, 2004). Genetic variability in tolerance to most environmental stress factors has been observed in both the legume host plants and their respective rhizobial strains (Hungria and Vargas, 2000; Serraj and Adu-Gyamfi, 2004), meaning that an effective chickpea host-rhizobia strain match should be researched to fit Ethiopian production.

The objective of this study was to evaluate the response of chickpea cultivars to commercial *Rhizobium* inoculant across three locations in the southern Ethiopia.

4.2 Materials and Methods

4.2.1 Experimental Procedures

The five chickpea cultivars and the three locations used in the previous trial (Chapter 3) were also used for this experiment. The three sites were described in Table 3-1.

The treatments were arranged in a factorial randomized complete block design with three replications at each location. A total of ten treatment combinations of five cultivars with rhizobium inoculation versus non-inoculation were randomized. The plot size was 3m x 4m. The plant spacing was 0.1 m between plants within a row and 0.3 m between rows resulting in a plant population density of 28 plants m^{-2}. The experiment was conducted for two consecutive years. In 2011growing season seeding was done on September 15 at Halaba, September 16 at Wolaita, and September 22 at Butajira. For the 2012 growing season, seeding was completed on September 15 at Butajira and September 16, at Halaba and Wolaita. The recommended rate of di-Ammonium phosphate (DAP) fertilizer was broadcasted on each block at a rate of 100 kg ha^{-1}. The N available in DAP (18 %) was considered sufficient as a starter N.

Seed for the inoculation treatment were treated with peat based inoculant of *Bradyrhizobium sp.* (*Cicer*) (BeckerUnderwood, Saskatoon, Canada).The ^{15}N natural abundance method was used to estimate biological N fixation and wheat (Triticum aestivum) cultivar Simba was used as the non-fixing reference crop for the experiments at all locations. One four row wheat plot was grown at the side of each replication. Manual weed control and cultivation were done for all plots. The 10 treatment combinations below were arranged in the manner as shown in Fig. 4-1.

No.	Cultivars	Treatments	
1.	Worku	T_1- Inoculated	T_2- without inoculation
2.	Natoli	T_3 - Inoculated	T_4- without inoculation
3.	Habru	T_5- Inoculated	T_6- without inoculation
4.	Ejere	T_7- Inoculated	T_8-without inoculation
5.	Ethiopian landrace	T_9- Inoculated	T_{10}-without inoculation

W	T_1	T_2	T_6	T_4	T_9	T_2	T_8	T_5	T_3	T_{10}

T=Treatments, W=Wheat,

Figure 4-1 Layout of one replication for the experiment showing treatment randomization

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4.2.2 Measurements of Agronomic and Physiological Parameters

Days to emergence was recorded for each plot when more than 50 % of the plants emerged. Similarly, days to flowering and days to maturity were recorded when more than 50 % of the plants in each plot attained flowering and 90 % at physiological maturity, based on yellowish color of the pods, respectively. At the time of maturity, five plants were randomly selected from the six middle rows and their height from the ground to the tip measured using a ruler. From the five randomly selected sample plants at maturity the number of pod bearing branches (both primary and secondary) were counted and averaged to give number of branches plant^{-1}.

Nodulation assessment was conducted at the pre-flowering stage. For all treatments, at pre-flowering, six plants were selected from the two border rows, three from each, and plants were dug at a depth of about 30 cm and carefully uprooted. The roots were washed with tap water to remove any adhering soil. The number of nodules per plant was counted and values averaged to give the number of nodules per plant. In addition, nodules were detached from roots, oven-dried at 70°C for 24 hours, then weighed to calculate nodule dry weight per plant.

During the harvesting time, data were recorded for yield components including number of pods per plant, number of seeds per pod, and hundred seed weight for each plot. For this purpose, five plants were randomly selected from the interior six rows. All of the middle six row plants were manually harvested and brought together on a plastic sheet for each plot. The harvested samples were sun-dried and measured to give weight of total biomass per plot. Then the plants were threshed manually for each plot. The grain was put in a cloth sack and weighed to give yield per plot which was later converted to tonnes ha^{-1}. On the same date, seeds were sampled and taken to the laboratory to measure their moisture content using a seed moisture meter (Model HOH-Express He 50, Germany). To keep uniformity, the final grain yield of each plot was adjusted to

12 percent seed moisture content. Harvest index (HI) was calculated after dividing the grain yield by biological yield (HI = grain yield / total above ground biomass).

Grain samples were taken from each treatment and ground using a cyclone mill to analyze nutrient concentrations in grain. . An acid digest of ground chickpea grain was conducted according to the method of Thomas *et al*. (1967). Between 250-300 mg of finely ground grain samples were weighed into glass digestion tubes and 5 ml of concentrated sulfuric acid (H_2SO_4) was added. Samples were placed on digestion block at 360°C for 30 min. Following this, samples were removed from the digestion block, allowed to cool, and 0.5 ml H_2O_2 was added. Samples were then placed on the digestion block an additional three times for 30 min, adding H_2O_2 after each heating period. Finally samples were placed on the digestion block for 1 hour. After samples were allowed to cool, distilled water was added to dilute the final volume of the sample to 75 ml to achieve a final concentration within the detection limit of the instrumentation. Then analysis for grain total nitrogen and phosphorus was done using an Auto-analyzer (Technicon™ auto-analyzer) colorimetry. The N value was multiplied by 6.25 to calculate the protein concentration. Grain Fe, Zn and Mg were analyzed using an Atomic Absorption Spectrophotometer at the University of Saskatchewan (AJ ANOVA 300, NY, USA). Similarly, dried grain samples were taken from each treatment and milled using a ball mill, reserved for ^{15}N natural abundance plant samples at the University of Saskatchewan. From each experimental unit, approximately 2 mg of ground seed sample was weighed into a tin capsule with standard weight (8×5 mm). The capsule was then closed, compressed and placed in 96-well micro plates. Samples were analyzed for natural abundance using a 20-20 Mass Spectrometer interfaced with an ANCA-GSL sample converter (Europa Scientific, Crewe, UK). The method known as the ^{15}N natural abundance (Peoples *et al*. 2009) is a widely used technique. The ratio of the two natural stable isotopic forms of N_2, ^{15}N: ^{14}N, can be measured by mass spectrometry. The natural ^{15}N abundance is therefore conventionally estimated as $\delta^{15}N$ values, where:

$$\delta^{15}N = \left(\frac{(^{15}N/\delta^{14}N \text{ Sample}) - (^{15}N/\delta^{14}N \text{ atmosphere})}{(^{15}N/\delta^{14}N \text{ atmosphere})}\right) \times 100$$

^{15}N values are expressed as parts per thousand or per mil (‰) with respect to the atmospheric N_2 gas (0.3663 atom % ^{15}N) (Peterson and Fry, 1987).

The percentage of N derived from the atmosphere via biological nitrogen fixation (%Ndfa) in chickpea grain was calculated as reported by Unkovich *et al*. (2008):

$$\% \text{ Ndfa} = \frac{\delta^{15}\text{N of reference plant} - \delta^{15}\text{N of } N_2\text{-fixing legume}}{\delta^{15}\text{N of reference plant} - \delta^{15}\text{N of } N_2} \times 100$$

The % Ndfa was calculated by comparing the $\delta^{15}N$ contents of the wheat sample with the chickpea samples:

Total nitrogen fixed per unit area by each cultivar was estimated following the equation:

$$\text{Nfix} = \text{NY} \times \text{Ndfa } \%$$

where NY represents the nitrogen yield, estimated with the following equation:

$$\text{NY} = \text{SY} \times \text{N } \%$$

where SY is seed yield and *N* % is the percentage of seed nitrogen in the sample.

Similarly protein yield (PY) per unit area was estimated with the following equation:

$$\text{PY} = \text{SY} \times \text{Protein } \%$$

where SY is seed yield and Protein % is the percentage of total protein in the seed sample.

4.2.3 Statistical Analysis

Data analysis of single environment and multi-environments was done using the PROC MIXED procedure of 9.3 SAS software (Little *et al*., 1996). For single environment analyses, the effect of replication was considered random whereas the effects of cultivar and inoculation were considered as fixed. In multi-environment analyses, the effects of cultivar, inoculation and cultivar x inoculation were fixed, and other variables were random. Least squared means (LSmeans) were computed for fixed effects using the LSMEANS statement. Standard errors of LSmeans were estimated and pair-wise tests of significant differences were performed using the PDIFF statement (at $p<0.05$ for significance).

4.3 Results

4.3.1 Soil Properties and Weather Data

Soil properties and weather data for each location are presented in Table 3-1 and 3-2 respectively. The soil at each location was a vertisol in Butajira, a clay loam in Wolaita and a sandy loam in Halaba. Soil pH ranged from 6.2-6.8 at 0-0.3 m depth. Total soil nitrogen concentration ranged from 61 to 71 kg ha^{-1} in the top 0.3 m depth and the N concentration was higher at Wolaita compared to Butajira and Halaba. Organic Carbon (OC %) content ranged from 1.61-1.99 percent in the 0-0.3 m depth.

Growing season (September to December in 2011 and 2012) precipitation in Wolaita (181-194 mm) and Butajira (100-126 mm) was higher than Halaba (56-74 mm). During the growing season, both Wolaita and Butajira received 45 % more cumulative rainfall than Halaba. Halaba experienced dry conditions in the month of October 2011 where no rainfall was recorded (Table 3-2). Similarly, December was dry for all three locations. Halaba had warm temperatures throughout the year experiencing an average daily temperature of greater than $20^{o}C$ (Table 3-2).

4.3.2 Agronomic Parameters

Results from the combined analysis (Table 4-1) indicated a highly significant ($p<0.001$) cultivar effect on branch number, pod number, grain Zn, Fe and Mg concentrations. Inoculation had non-significant effects on yield and yield components. The cultivar x inoculation interaction effect was highly significant ($p<0.001$) for plant height only.

The cultivar by environment interaction effect was significant for N fixation and agronomic traits such as days to 50 % flowering, maturity, 100-seed weight, harvest index and grain yield hence results are presented separately. Analysis for N fixation was done using the natural abundance methods. Transformation of the N raw data was done to achieve homogeneous error variances. High variability in nitrogen fixation occurs due to the influence of environment therefore result of test environments is presented separately (Table 4-2).

Table 4-1 Analysis of variance (mean, standard errors and p-values) for days to flowering, days to maturity, plant height, branch number, yield and yield components of five chickpea cultivars (C) under inoculation and non-inoculation treatment (I) across environments (5 site-year [SY]) in southern Ethiopia.

	Days to Flower	Days to Maturity	Plant Height (cm)	Branch No	Pod No	100 Seed Weight (g)	Harvest Index	Yield (t ha^{-1})
Cultivar (C)	0.001	0.023	0.36	0.001	0.001	0.001	0.16	0.009
Inoculation (I)	0.25	0.37	0.53	0.96	0.59	0.83	0.25	0.38
C * I	0.17	0.12	0.001	0.88	0.49	0.47	0.73	0.13
Site year (SY)	0.06	0.06	0.06	0.06	0.06	0.07	0.15	0.07
SY * C	0.004	0.001	0.17	-	0.28	0.042	0.034	0.006
SY * I	-	0.33	-	-	0.24	-	0.38	0.41
SY * C * I	0.023	-	-	0.09	-	0.46	-	-
Mean	40.1	102	36.7	8.3	50.1	25.7	0.5	1.93
SE	0.68	0.89	0.52	0.02	0.02	0.68	0.01	0.05

Significant at $p<0.05$ level

Table 4-1 Analysis of variance (mean, standard errors and p-values) for, protein and grain nutrient concentrations of five chickpea cultivars (C) under inoculation and non-inoculation treatment (I) across environments (5 site-year [SY]) in southern Ethiopia (Continued)

	Protein %	P (mg g^{-1})	Mg (mg g^{-1})	Fe (mg g^{-1})	Zn (mg g^{-1})
Cultivar (C)	0.69	0.43	0.001	0.006	0.076
Inoculation (I)	0.38	0.64	0.52	0.47	0.83
C * I	0.58	0.31	0.59	0.16	0.53
Site Year	-	0.31	-	-	-
SY * C	-	-	-	-	-
SY * I	-	-	-	-	-
SY * C * I	0.26	-	-	0.37	-
Mean	16	2.84	1.38	0.546	0.041
SE	0.21	0.057	0.011	0.001	0.005

Significant at $p<0.05$ level

Table 4-2 Analysis of variance (p-values) for nodule number, nodule dry weight, nitrogen fixation, total nitrogen and protein yield of five chickpea cultivars (C) under inoculation and non-inoculation treatment (I) at three locations in southern Ethiopia in 2011 and 2012

Year / Location	Treatment	Nodule No.	Nodule Dry weight (mg)	% Ndfa	Total Fixed N (kg ha^{-1})	Total N Yield (kg ha^{-1})	Total Protein Yield (kg ha^{-1})
2011 Wolaita	Cultivar (C)	0.120	0.230	0.832	0.064	0.001	0.001
	Inoculation (I)	0.195	0.551	0.215	0.478	0.079	0.079
	C * I	0.816	0.965	0.403	0.201	0.893	0.893
Butajira	Cultivar (C)	0.002	0.001	0.296	0.049	0.001	0.001
	Inoculation (I)	0.947	0.729	0.466	0.477	0.606	0.606
	C * I	0.461	0.067	0.887	0.782	0.844	0.844
2012 Wolaita	Cultivar (C)	0.051	0.001	0.022	0.001	0.001	0.001
	Inoculation (I)	0.258	0.157	0.955	0.926	0.910	0.910
	C * I	0.692	0.214	0.386	0.270	0.135	0.135
Butajira	Cultivar (C)	0.216	0.192	0.521	0.794	0.001	0.001
	Inoculation (I)	0.372	0.135	0.177	0.122	0.001	0.001
	C * I	0.379	0.619	0.255	0.226	0.101	0.101
Halaba	Cultivar (C)	0.964	0.445	0.039	0.085	0.487	0.487
	Inoculation (I)	0.659	0.376	0.944	0.594	0.148	0.148
	C * I	0.798	0.576	0.129	0.323	0.220	0.220

Significant at $P<0.05$ level

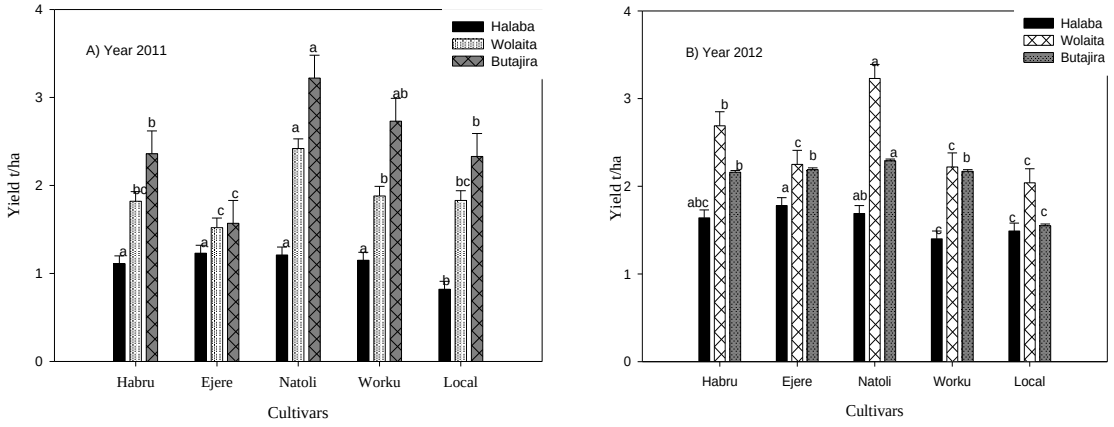

Figure 4-2 Grain yield (tonnes ha^{-1}) of five chickpea cultivars in Halaba, Wolaita and Butajira in 2011 (A), and 2012 (B) inoculation trial. Bar graphs with the same letter in the same year at each location are not significantly different at P<0.05

Overall grain yield of chickpea cultivars significantly varied over years and locations (Fig. 4-2). Cultivar Natoli ranged from 1.2 – 3.2 tonnes ha^{-1} and constantly ranked as the best cultivar for yield across locations in 2011, followed by Worku (1.1 – 2.7 tonnes ha^{-1}). Similarly, in 2012 Natoli had higher yield (1.7 – 3.2 tonnes ha^{-1}) followed by Habru (1.6 – 2.7 tonnes ha^{-1}). Although inoculation of chickpea with *Rhizobium* inoculant did not significantly influence grain yield across environments, individual location analysis in 2012 revealed significant effect of inoculation on grain yield at Halaba and Butajira.

The interaction effect of cultivar by inoculation revealed taller local landrace chickpea plants (39 cm) over the uninoculated treatment (35 cm). Other cultivars like Ejere and Natoli had a similar height to each other whereas cultivars Habru and Worku were shorter than uninoculated plants.

Table 4-3 Pod number, branch number and nodule dry weight of five chickpea cultivars averaged across environment

Cultivar	Pod Number Plant^{-1}	Branch Number Plant^{-1}	Nodule Dry Wt. (mg) Plant^{-1}
Habru	42^{cd}	7b^{c}	12^{bc}
Ejere	39^{d}	7^{c}	12.3^{bc}
Natoli	48^{c}	8^{b}	13.5^{a}
Worku	57^{b}	11^{a}	13.2^{ab}
Landrace	72^{a}	9^{a}	11.7^{c}
Mean	50	8	12.4
SE	0.08	0.12	0.02

Means followed by the same letter(s) within a column are not significantly different based on LSD at $p < 0.05$

Number of branches plant^{-1} was significantly ($p<0.05$) different across cultivars (Table 4-3). The maximum number of branches (11) was produced by Worku followed by the local landrace (9). However, numbers of branches between inoculated and non-inoculated plants were the same (Table 4-1). Cultivars significantly varied for the number of pods plant^{-1}. Accordingly, the highest number of pods (72) was recorded for the local landrace whereas the lowest (39) was recorded for Ejere (Table 4-3). Nodule dry weight ranged from 11-13 mg plant^{-1} (Table 4-3). The low weight of nodule might be due to small size and number of nodules per plant.

The weight of hundred seeds varied significantly ($p<0.05$) by the cultivars and environment. The maximum mean weight of hundred seeds was recorded for Ejere (36 g) in 2011 and 35 g in 2012, followed by Habru. The local landrace had the lowest hundred seed weight (13 g) in both years (Fig.4-3).

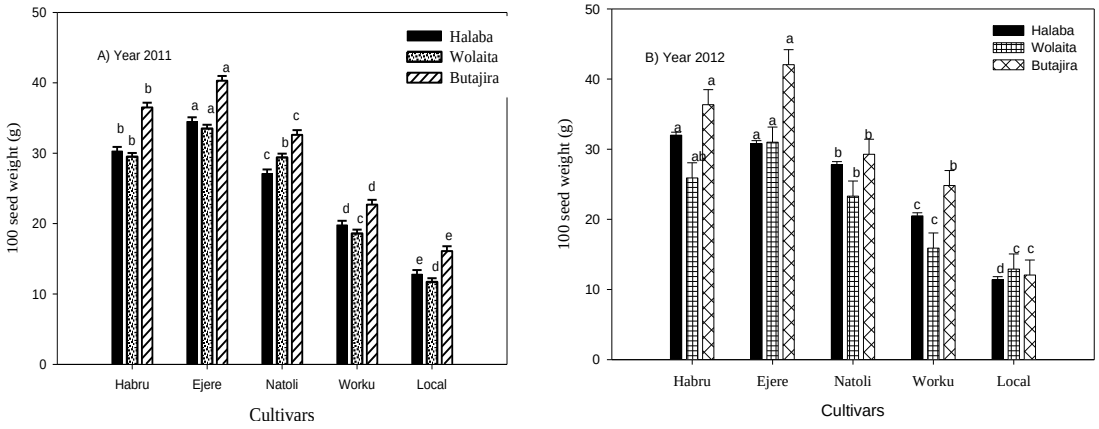

Figure 4.3 100-seed weight of five chickpea cultivars in Halaba, Wolaita and Butajira in 2011 (A), and 2012 (B). Bar graphs with the same letter in the same year at each location are not significantly different at $p<0.05$

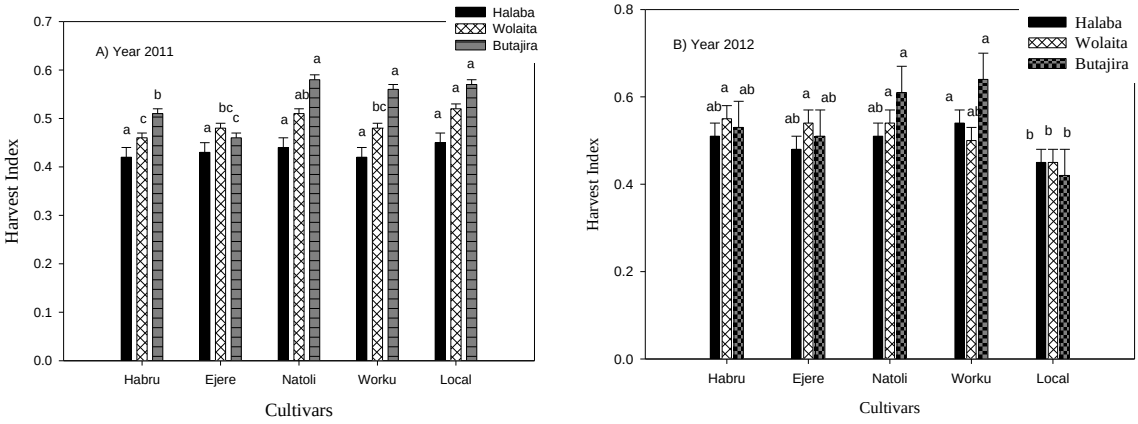

Figure 4-4 Harvest index of five chickpea cultivars in Halaba, Wolaita and Butajira in 2011(A), and 2012 (B). Bar graphs with the same letter in the same year at each location are not significantly different at $p<0.05$

In 2011 Natoli and Ethiopian landrace had greater harvest index (Fig. 4-4) in all the three locations (A) but in 2012 the improved cultivars had greater harvest index than the Ethiopian landrace (B).

4.3.3 Nitrogen Fixation

4.3.3.1 Percentage of Nitrogen Derived from Atmosphere (% Ndfa)

There were no significant differences in percent nitrogen derived from the atmosphere between inoculated and non-inoculated treatments across all environments (Table 4-2 and 4-4). Significant variation was detected for nitrogen derived from atmosphere (% Ndfa) across cultivars in Halaba and Wolaita in 2012 (Table 4-2). Cultivars had a range of 26 to 48 % Ndfa in a less moisture environment (Halaba 2012) and 42 to 54 % Ndfa in a moist (Wolaita 2012) environment (Table 4-2). No significant difference for % Ndfa was observed among cultivars at Wolaita and Butajira in 2011 and at Butajira in 2012. Natoli and Ethiopian landrace in Halaba 2012, and Natoli and Worku in Wolaita 2012 all had higher % Ndfa. Habru and Ejere had an average 26 % Ndfa in Halaba 2012 and 42 % in Wolaita 2012 which were the lowest % Ndfa, compared to other cultivars.

4.3.3.2 Nitrogen Fixed per unit Area

There were significant differences among cultivars for nitrogen fixed per unit area (Kg ha^{-1}) in Butajira 2011 and Wolaita 2012 (Table 4-2). In Butajira 2011, Natoli (57 kg ha^{-1}) had more N fixed per unit area, about 171 % greater than Ejere ($p = 0.04$). In Wolaita 2012 Natoli (52 kg ha^{-1}) had higher fixed nitrogen per unit area than Ethiopian landrace (29 kg ha^{-1}) and Ejere (29 kg ha^{-1}). Among the cultivars, Ejere fixed less nitrogen per unit area than all other cultivars in all environments in 2011 and 2012 (Table 4-4). There was no significant difference on N fixed per unit area among cultivars in Wolaita 2011, and Halaba and Butajira in 2012 (Table 4-2 and 4-4).

4.3.3.3 Nitrogen Yield per unit Area

Grain nitrogen yield per unit area was different among cultivars ($p< 0.05$) in all environments except in Halaba 2012 (Table 4-2 and 4-5). In Halaba and Butajira 2012 a significant difference between inoculated and non-inoculated cultivars was observed, where inoculation increased nitrogen yield per unit area by an average of 14 percent. Natoli had consistently more nitrogen yield per unit area across all environment and years.

4.3.3.4 Protein Yield per unit area

Rhizobium inoculation increased the protein yield (Kg ha^{-1}) of chickpea cultivars by about 13 % and 15 % in Halaba and Butajira 2012, respectively (Table 4-5). A significant difference for protein yield per unit area was observed among cultivars ($p < 0.05$) in all environments except in Halaba 2012 (Table 4-2 and 4-5). High protein yield was harvested consistently from Natoli across all environment and years. The Ethiopian landrace had the lowest protein yield per unit area in most environments.

4.3.3.5 Nodule Weight and Number

The assessment of nodule number per plant was carried out at late vegetative stage, where nodule formation is expected to be maximum. All treatments had no significant ($p=0.604$) effect on nodule number (Table 4-2 and 4-4). The average nodule numbers (12 nodules) are relatively low as compared to other research findings. Nodule number in chickpea can be affected by several factors such as soil moisture, rhizobium inoculation and N fertilizer. Fertilizer N and soil moisture had a significant, negative effect on the number of nodules formed on the chickpea roots. On average, application of 40 N (at 60 % FC) decreased the number of nodules to 93 $plant^{-1}$ and 200 nodules $plant^{-1}$ at 20 N (60% FC) compared to 226 at the 0 N (at 60 % FC) control (Gan *et al.,* 2008). El Hadi and Elsheikh (1999) reported rhizobium inoculation significantly increased nodule number of six chickpea cultivars with an average nodule number ranging 20-29 whereas N fertilization (50 kg N ha^{-1}) and no inoculation resulted in zero nodule number $plant^{-1}$. Natoli had the maximum nodule number (15), Ejere and Local landrace had similar number of 10 nodules.

Nodule dry weight was significantly different among cultivars (Table 4-3). Cultivars Natoli and Worku had a similar but greater nodule dry weight (13 mg) whereas local landrace had 11 mg. The relatively low nodule dry weight could be due to smaller nodule size and number that might be caused by moisture stress (Appendix 7).

Table 4-4 Comparison among five chickpea cultivars for nitrogen fixation traits in the field trials in Southern Ethiopia, 2011-2012

Treatment	Percentage of Nitrogen derived from the atmosphere (% Ndfa)					Total Nitrogen Fixed (Kg ha^{-1})				
	2011			2012		2011			2012	
	Wolaita	Butajira	Halaba	Wolaita	Butajira	Wolaita	Butajira	Halaba	Wolaita	Butajira
Inoculation										
Inoculated	46.7	46.6	33.6	46.9	28.9	31.9	35.8	19.6	35.9	18.9
Non-inoculated	48.5	51.7	33.3	39.1	20.4	30.8	40.8	17.2	29.4	11.2
P-value	0.215	0.466	0.944	0.955	0.177	0.478	0.477	0.594	0.270	0.122
Cultivars										
Natoli	39.0	55.9	48.3^{a}	54.4^{a}	20.8	35.7	56.8^{a}	28.6^{a}	51.6^{a}	13.5
Landrace	52.4	58.2	40.4^{ab}	44.7^{b}	31.9	33.8	46.4^{a}	21.4^{ab}	28.8^{bc}	14.2
Worku	49.9	37.7	28.8^{b}	48.7^{ab}	24.8	33.0	34.1^{ab}	15.2^{b}	33.2^{b}	15.8
Habru	44.1	52.1	26.8^{b}	41.9^{b}	29.9	27.5	37.9^{ab}	14.2^{b}	35.4^{b}	17.6
Ejere	52.2	43.3	25.8^{b}	42.4^{b}	16.5	26.8	21^{b}	14.9^{b}	28.6^{c}	10.2
P-value	0.832	0.296	0.039	0.022	0.521	0.064	0.049	0.085	0.001	0.794

Means within a column and treatment, (Inoculation or Cultivars) followed by the same letters are not significantly different ($p < 0.05$).

Table 4-5 Comparison among five chickpea cultivars for nitrogen fixation related traits in the field trials across three locations in southern Ethiopia, 2011-2012

Treatment	Total Nitrogen yield (Kg ha^{-1})						Total Protein yield (Kg ha^{-1})					
	2011			2012			2011			2012		
	Halaba	Wolaita	Butajira	Halaba	Wolaita	Butajira	Halaba	Wolaita	Butajira	Halaba	Wolaita	Butajira
Inoculation												
Inoculated	34.1	69.9	76.5	58.5^{a}	76.6	62.8^{a}	213	437	478	366^{a}	479	393^{a}
Non-inoculated	32.5	62.8	80.2	51.6^{b}	75.2	54.7^{b}	203	397	501	323^{b}	470	342^{b}
P-value	0.52	0.08	0.61	0.033	0.71	0.001	0.52	0.08	0.61	0.033	0.71	0.001
Cultivars												
Natoli	36.6^{a}	86.2^{a}	102^{a}	59.3	94.8^{a}	64.7^{a}	229^{a}	539^{a}	641^{a}	371	592^{a}	405^{a}
Landrace	24.6^{b}	63.6^{bc}	80.2^{ab}	51.9	64.6^{b}	44.4^{c}	154^{b}	397^{bc}	501^{ab}	325	404^{b}	278^{c}
Worku	33.8^{a}	67.3^{b}	89.2^{ab}	52.8	68.2^{b}	63.9^{a}	211^{a}	420^{b}	557^{ab}	330	426^{b}	400^{a}
Habru	36.4^{a}	63.5^{bc}	72.8^{b}	53.1	84.4^{b}	58.8^{b}	228^{a}	397^{bc}	455^{b}	332	528^{a}	368^{a}
Ejere	34.8^{a}	51.5^{c}	46.9^{c}	58.1	67.5^{b}	62^{ab}	218^{a}	322^{c}	294^{c}	363	422^{b}	388^{ab}
P-value	0.032	0.003	0.001	0.424	0.001	0.001	0.032	0.003	0.001	0.424	0.001	0.001

Means within a column and treatment, (Inoculation or Cultivars) followed by the same letters are not significantly different ($p< 0.05$).

4.4 Discussion and Conclusion

Combined analysis of variance indicated non-significant effect inoculation treatment on all parameters. Such inoculation failure might be associated with the environmental conditions and management practices. The primary environmental factor that may have contributed to inoculation failure is dry seedbed condition, a common occurrence in semi-dry areas of the southern Ethiopia. Early growing season of chickpea in 2011was affected by moisture stress due to low precipitation that resulted in dry, warm soils that may have desiccated and destroyed rhizobium cells before the root infection could occur. Nodule number and nodule dry weight was better in 2012 compared to 2011. The effects of environmental conditions on Rhizobium inoculation have previously been reported (McConnell, *et al*., 2001; Serraj, 2004; Gault, *et al*., 1984). Poor number of nodules and low nodule dry weight can be considered as evidence for inoculation failure possibly due to moisture stress. Another possible reason for the cause of inoculation failure was adverse storage conditions of the inoculant during the transportation from Canada to Ethiopia, and also the possibility of incompatibility of rhizobial strain and the current chickpea cultivars.

Research reports indicated that success of inoculation depends on the environmental conditions, soil fertility (Bottomley, 1992; Graham, 1992), number and application method of effective rhizobial cells (Brockwell and Bottomley, 1995; Brockwell *et al*., 1995), presence of high populations of competing strains of rhizobia (Thies *et al*., 1991), and lastly, plant genotype (Hafeez *et al*., 1998).The study areas were known to often experience moisture stress and the soils were less fertile. Such conditions may have contributed to the yield variations across location and years. The current research only tested one strain of chickpea rhizobia. A more research on a range of strains is needed to identify the best strain for the current adapted cultivars.

The 2011 growing season received less rainfall compared to 2012 (Table 3-2). Halaba and Butajira experienced moisture stress in the first 2-3 weeks of the vegetative growth stages in 2011. This could be the reason for the failure to observe the effect of inoculation in 2011. Kantar (2010) reported the onset of drought at the vegetative phase of grain legumes would adversely affect the plant nitrogen fixation response in comparison with drought at the reproductive phase. Other reports also indicated a differential effect of rhizobium strains with significant differences

in their survival rate under rainfed and dry land situations exhibiting variable moisture stress conditions in chickpea (Kantar *et al*.,2003), and in lentil (Athar, 1998).

Despite a low total soil nitrogen concentration, and a nominal indigenous population (< 10 gram^{-1} of soil) of resident rhizobia (Ibsa, 2013) in Wolaita, seed yield did not respond to inoculation in 2011 and 2012. This could also be due to an adaptation problem. It is possible that the commercial strain of chickpea inoculant used in this study was poorly adapted to this soil-climatic region; this is evidenced by the poor number of nodules and less efficient nodules. This has been previously reported by other researchers with commercially available chickpea inoculants in new chickpea production areas (Walley *et al.*, 1997; McConnell, *et al*., 2001) where they found no significant difference between inoculated and uninoculated plants for shoot N and biomass before anthesis in chickpea.

High short-range spatial variability in N_2 fixation has been demonstrated by Walley *et al.* (2001). In a field study, BNF of chickpea (*Cicer arietinum* L.) was measured at 0.3 m intervals on a 33 m transect, using wheat (*Triticum aestivum* 'Katepwa') as reference crop. Each crop was sampled at 110 points along transect. Estimates of BNF in the grain varied from 36 to 70 %, with a mean value of 55 % (Walley *et al.*, 2001).Therefore, separate analysis over location and years was done to have a reliable estimate of N fixation in this thesis.

Inoculation of chickpea cultivars did not show significant effect on % Ndfa; however cultivars differ significantly for % Ndfa across locations and years. Similar results were reported by Latif *et al*. (2014). The current % Ndfa results are consistent with soil moisture and nutrient deficiency affecting the process of nitrogen fixation through direct and indirect effects on nitrogenase functioning and on plant growth, respectively. Ibsa (2013) reported a significant effect of combining rhizobium inoculant with P on grain yield in the Wolaita area. As expected with late maturing chickpea cultivars, Natoli had more % Ndfa (54%) in Wolaita and Halaba 2012. Increased nodule dry weight of Natoli may have contributed to the higher % Ndfa. In contrast, Habru and Ejere, cultivars being early maturing, had lower % Ndfa and nodule dry weight compared to Natoli. Similar findings were reported by Gan *et al*. (2010) on higher % Ndfa associated with increased nodule dry weight.

In this study low N fixation of inoculated chickpea could also be due to poor nodulation as observed from the number of nodules per plant. This is consistent with previous N fixation reports of (Elias *et al*., 2004; Deaker *et al*., 2004; Elias, 2009) where poor nodulation occurred due to poor adhesion of rhizobia to the seed using the inoculation technique of dry peat on seed. Elias (2009) added that careful consideration, however, should be given to the fact that the presence of nodules does not necessarily translate to high N fixation. Optimum productivity of chickpea in southern Ethiopia can be achieved by applying effective viable rhizobium inoculants using proper technique.

The number of nodules was similar for all cultivars, but nodule weight was greater for Natoli and Worku (Table 4-3). Because % Ndfa was better for Natoli (54 %) compared to Ejere (26 %), this may imply that weight of nodules may be more important for fixation than their number. Better N fixation at Wolaita in 2012 than Halaba may also be specifically attributed to the higher level of moisture in the soil since moisture is of paramount importance in fixation. Gemechu (2012) also found nodule dry weight contributed to greater nitrogen fixation.

These findings of less effective inoculation than desired highlight the difficulty in identifying the potential causes of variable response to inoculation and the resulting poor N_2 fixation. The result also implied that, there is a need to evaluate the inhibitive factors for N fixation in the region and to develop potential solutions such as identification of effective strain of rhizobia that can improve nodulation and N fixation for a range of host cultivars, in the region.

5. Preface

In southern Ethiopia farmers traditionally practice a cereal-cereal crop rotation. The continuous cropping with minimal or no fertilizer input has contributed to the depletion of soil N resulting in low yield. The potential to grow chickpea as part of crop rotation in the region with the application of an appropriate strain of N fixing bacteria, either to the seed or to the soil will provide an opportunity for the famers in the region. The use of inoculation technology for chickpea in a crop rotation system will help to reduce some production issues such as low fertilizer input, and to sustain farming practices. The positive effects of crop rotation can be seen in different aspects of crop production. The next research (Chapter 5) focused on chickpea-wheat rotation. The research assessed the benefits of chickpea N residue for succeeding wheat yield and soil N.

Five chickpea (*Cicer arietinum* L.) cultivars were inoculated and studied along with a control (un-inoculated) to determine the effect of inoculum on N fixation and crop yield (Chapter 4). Wheat (*Triticum aestivum* L.) cv. Simba was seeded during the summer and harvested in the fall of 2012. This chapter also discussed the nitrogen use efficiency (NUE) in this cropping system.

5. Agronomic Performance of Wheat in Chickpea-Wheat Rotation

5.1 Introduction

In rainfed tropical environment cereal yields are usually low and unpredictable due to poor soil fertility. Development of cropping systems that are able to efficiently use water and nitrogen are essential to maximize yield, reduce production costs and environmental pollution through poor N fertilizer uptake. Chickpea production works well in rotation with cereals such as wheat (*Triticum aestivum*) and teff (*Eragrostis tef),* which are widely grown in relatively well-drained black soils of southern Ethiopia. Chickpea is typically seeded at the end of the main rainy season, the end of September, using residual soil moisture. This allows farmers to practice double cropping, where the main crop maize or wheat is planted from March to August followed by chickpea from September to December. This double cropping approach increases the land productivity per unit time and provides an additional source of income for farmers (Menale *et al*., 2009).

Contribution of legumes towards the N economy in cereal-based cropping systems is well-known (Sharma and Behera, 2009; van Kessel and Hartley, 2000; Greenland, 1971). In crop rotations, grain legumes contribute to diversification of cropping systems and as N_2-fixing plants they can reduce mineral N fertilizer demand (Mayer *et al*., 2003). Generally, in sustainable and organic farming systems, biological N_2-fixation by legumes is used as the main source of nitrogen for the succeeding crop. Hence cropping systems with legumes are a priority area of research in rainfed or dryland production.

Grain legumes usually provide positive yield effects on the subsequent non-legume crops when compared with rotations containing non-legumes (Chalk, 1998; Sanginga, 2003). In addition to its beneficial factors, such as improving soil structure, breaking pest and disease cycles and the phytotoxic and allelopathic effects of crop residues, nitrogen is a key factor in the positive response of cereals following legumes (Chalk, 1998). However the improvement in N nutrition of non-fixing crops in grain legume-based cropping systems requires a more fundamental understanding of the decomposition processes of grain legume residues and their interactions with soil organic matter. Grain legume species and cultivars growing at the same location differ significantly in dry matter production, N accumulation, N_2-fixation, N-balance and residue

quality (Beck *et al*., 1991). These differences may be the main factors determining the residual N contribution to subsequent crops (Hood *et al*., 1999).

The inclusion of legumes in crop rotation increases soil fertility and consequently the productivity of succeeding cereal crops (Ghosh *et al*., 2007). Kumar and Prasad (1999) reported a saving of 25 kg N ha^{-1} in wheat when grown after a grain legume. The nitrogen economy was affected not only due to direct N addition through legume residues and its subsequent mineralization but also due to enrichment of soil with fixed N_2 from root exudates (Pawar and Jadhav, 1995). Such information is lacking for the production system in the rainfed areas of Halaba and Wolaita in the southern Ethiopia. This research examined the rotational effects of chickpea cultivars under inoculation and non-inoculation at two sites in Halaba and Wolaita of Southern Ethiopia, where the chickpea in 2011 fall season was followed by wheat grown in the summer season the following year.

Various aspects of N economies and N benefits of chickpea have been quantified in different studies (Pawar and Jadhav, 1995; Sharma and Behera, 2009; Hayat and Ali, 2010). But much of the research on chickpea deals with systems in which above-ground plant residues are retained in the soil. In Ethiopia chickpea residues are commonly harvested together with the grain and used for fuel and fodder. Therefore Ethiopian production represents a more N user-intense system than previous published research.

The objective of this study was to determine the residual N effect of inoculated chickpea cultivars on soil mineral nitrogen, straw yield, and grain yield of wheat in a chickpea wheat rotation with some or no N fertilizer application in southern Ethiopia (Halaba and Wolaita).

5.2 Materials and Methods

The experiment was conducted at two sites, Halaba and Wolaita, where chickpea cultivars were seeded under inoculation and non-inoculation treatments (Chapter 4). An early maturity bread wheat (*Triticum aestivum*) cv. Simba was used for the evaluation of residual effect of nitrogen on wheat in a chickpea-wheat rotation system. Seeding was done on July 24, 2012 in Halaba and July 27, 2012 in Wolaita. The design was a split-split plot with three replications and two locations. Factors include five chickpea cultivars as the main plot (3m x 4m), and with and without rhizobium inoculation as the sub plot (3m x 4m), and inorganic N fertilizer as the sub-sub plot (3m x 2m). Two rates of fertilizer, 0 kg ha^{-1} urea and 50 % of the recommended urea nitrogen rate (78 kg ha^{-1} Urea) were applied to each sub plot on the previously inoculated and non-inoculated chickpea harvested field at each of the locations. Bulk soil samples were taken before seeding and after wheat harvest from each plot at a depth of 0-0.3 m for nitrogen analysis. Harvesting of wheat was done on November 12, 2012 in Halaba and December 7, 2012 in Wolaita.

5.2.1 Measurements of agronomic and physiological parameters

Days to emergence and physiological maturity were recorded on a plot basis. At the time of maturity, five wheat plants were randomly selected from each plot and their heights from the ground surface to the top of the terminal spikelet were measured using a ruler, for average height. Harvesting was done from one meter square area of each plot. The harvested samples were sun-dried and weighed for total biomass per plot. Then the plants were threshed manually for each plot. The grain was put in a cloth sack and weighed to give yield per plot and converted to tonnes ha^{-1}. Seed samples of harvested plants in each plot were used to measure 100-seed weight. For uniformity, final grain yield of each plot was adjusted to 12 % seed moisture content. Harvest index (HI) was calculated after dividing the grain yield by biological yield (HI = grain yield / total above ground biomass).

An acid digest of ground chickpea grain was conducted according to the method of Thomas *et al*. (1967). Between 250-300 mg of finely ground grain samples were weighed into glass digestion tubes and 5 ml of concentrated sulfuric acid (H_2SO_4) was added. Samples were placed on digestion block at 360°C for 30 min. Following this, samples were removed from the digestion

block, allowed to cool, and 0.5 ml H_2O_2 was added. Samples were then placed on the digestion block an additional three times for 30 min, adding H_2O_2 after each heating period. Finally samples were placed on the digestion block for 1 hour. After samples were allowed to cool, distilled water was added to dilute the final volume of the sample to 75 ml to achieve a final concentration within the detection limit of the instrumentation. Then analysis for grain total nitrogen and phosphorus was done using an Auto-analyzer (Technicon™ auto-analyzer) colorimetry. The N value was multiplied by 6.25 to calculate the protein concentration.

The export of N with the harvest of wheat grain and mineral N fertilizer are the major flows in and out of this system. The ratio between harvested N and applied N can therefore be used to describe the efficiency of N fertilizer utilization in crop production. For the present study, Nitrogen Use Efficiency (NUE) has been calculated according to following equation (Raun and Johnson, 1999).

NUE = (Total Cereal N removed) - (N coming from the soil) / N applied as fertilizer to soil) * 100

Where:

Total N removed = Grain yield of wheat x average N concentration

N coming from soil = The control treatment or 0 kg N applied

N fertilizer applied = Mineral N input

5.2.2 Statistical Analysis

Data analysis was done using the PROC MIXED procedure of 9.3 SAS software (Little *et al*., 1996). Multi-environment data were subjected to an analysis of variance in mixed model. The effects of replication, replication within location, and the interaction effects with replication and location were considered random to fit a split-plot analysis, while the effects of cultivar, inoculation, fertilizer and their interaction effects were considered fixed. Least squared means (LSmeans) were computed for fixed effects using the LSMEANS statement. Standard errors of LSmeans were estimated and pair-wise tests of significant differences were performed using the PDIFF statement at $P<0.05$.

5.3 Results

5.3.1 Nitrogen Effect Benefit of Chickpea Rotation

Total soil N content after chickpea was increased (0.24 % N) on average than before chickpea seeding (0.16 % N). No significant change in the total soil N value was found due to inoculation (N fixation), since inoculation was ineffective (Chapter 4).

Table 5-1 Total nitrogen (average of two sites) in the soil before chickpea seeding (2011), after chickpea harvest before wheat, and after wheat harvest in the following calendar (2012)

Total Soil N (%) (at 0-0.3 m depth)			
Before planting chickpea		After chickpea harvest	After wheat harvest
0.16	Landrace	0.27[a]	0.18[a]
	Habru	0.26[a]	0.18[a]
	Ejere	0.24[a]	0.17[a]
	Worku	0.23[a]	0.15[a]
	Natoli	0.23[a]	0.17[a]
	Mean	0.24	0.17

Means followed by the same letter within column did not differ at $p<0.05$

Results from soil sample analysis taken before wheat seeding (7 month after chickpea harvest), and after wheat harvest, showed a significant difference in soil N available (Table 5-2). The previous crop history in the Wolaita experimental site was maize, teff, wheat and potato and at Halaba the non-legume perennial tree called chat (*Khata edulis*) was cropped for the prior 6-8 years. Initial total soil N was low (0.16 % N) but seeding chickpea increased the soil total N by 56 % (0.24 % N). Soil analysis after wheat grown in the following year indicated that the crop has used most of the available N in the soil, leaving a remaining N content slightly greater than the initial soil N.

Table 5-2 Rotation effect of chickpea on total soil nitrogen compared with continuous non-legume cropping.

	Rotation effect[1]
	Total Soil N (%) (at 0-0.3 m depth)
Initial soil N	0.16
Chickpea - without inoculation[2]	0.25[a]
Chickpea - with inoculation[2]	0.24[a]
Chickpea-wheat (plot without inoculation)[2]	0.17[b]
Chickpea-wheat (plot with inoculation)[2]	0.17[b]

[1] Improvement of total soil N in the rotation-compared to initial soil N before chickpea
[2] Within inoculation and chickpea-wheat rotation total soil N mean. Different letters indicate significant differences at $p<0.05$

5.3.2 Residual Effect of Chickpea on Succeeding Wheat Yield and Yield Attributes

Wheat after five chickpea cultivars, inoculated and un-inoculated with rhizobia in both sites produced significantly greater yield, straw yield and plant height mainly due to the application of N fertilizer. Neither cultivars nor inoculation had significant influence on wheat yield (Table 5-4). The interaction of inoculation by fertilizer treatment significantly affected grain P concentration.

The results showed that maximum wheat grain yield of 2.25 tonnes ha^{-1} was obtained in the treatment of chickpea-wheat with fertilizer-N, which had 19 % increase over control i.e., chickpea-wheat with no fertilizer N. Total above ground biomass yield of wheat was highest (3.45 tonnes ha^{-1}) in plots with fertilizer-N (Fig. 5-1). This yield was 26 % more as compared to the biomass yield (2.73 tonnes ha^{-1}) of the control of chickpea- wheat with no fertilizer-N. Nitrogen had a highly significant ($p<0.001$) effect on plant height.

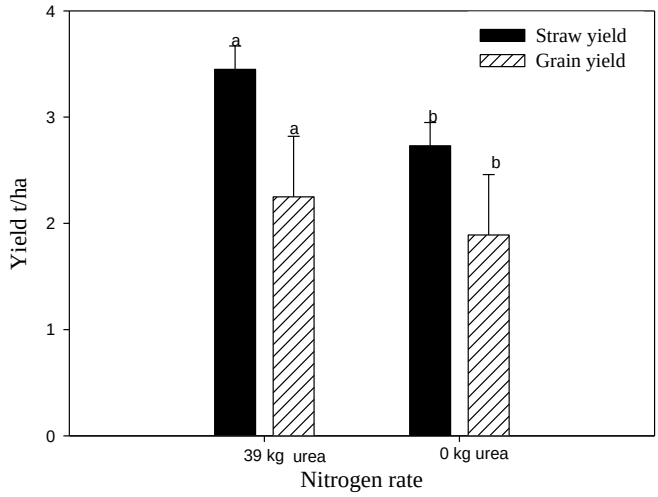

Figure 5.1 Effect of N fertilizer and chickpea-wheat rotation on straw and grain yield of wheat in 2012 Halaba and Wolaita trials

5.3.3 Nitrogen Use Efficiency (NUE)

NUE provides information about the relative utilization of additional N applied to an agricultural production system of a certain area. N use efficiency (NUE) of 30.5 % was considered low resulting in soil N loss and yield reduction in this study (Table 5-3).

Table 5-3 Nitrogen Use Efficiency (NUE) on a fertilizer application basis of wheat grain at two rates of N fertilizer

Applied N (Kg ha^{-1})	Grain yield (Kg ha^{-1})	Grain N concentration (%)	N uptake (Kg ha^{-1})	NUE
0	1898	1.67	31.7	
18	2254	1.65	37.2	30.5

Table 5-4 Mean and p-values of wheat agronomic characteristics under chickpea-wheat rotation trial in Halaba and Wolaita 2012

Source of variation	Days to Maturity	Plant height (cm)	100 Seed weight (g)	Harvest Index	Straw Yield (t ha^{-1})	Grain Yield (t ha^{-1})	Grain Protein (%)	Grain P (mg g^{-1})
Main plot (V=Cultivar)	0.95	0.92	0.72	0.33	0.18	0.86	0.69	0.15
Sub plot (IN=inoculation)	0.4	0.99	0.29	0.53	0.17	0.99	0.65	0.29
V * IN	0.89	0.20	0.44	0.66	0.42	0.47	0.66	0.10
Sub-sub plot(F=Fertilizer)	1	0.001	0.65	0.54	0.003	0.001	0.09	0.24
V * F	1	0.85	0.38	0.22	0.72	0.82	0.23	0.18
IN * F	1	0.89	0.26	0.48	0.15	0.43	0.42	0.018
V * IN * F	1	0.64	0.62	0.54	0.89	0.82	0.24	0.46
Mean	106	58.2	3.85	0. 4	3.09	2.07	10.4	4.08
SE	0.612	0.73	0.032	0.01	0.101	0.076	0.067	0.071

Where; V= Cultivars (Main plot); IN= Inoculation –with and without inoculation (Sub plot); F= N Fertilizer-0 and 50 % (Sub-sub plot); Significant at $p<0.05$

5.4 Discussion and conclusions

Soil total N was low (0.16 % N) before chickpea was seeded. Similar low soil N was reported in Halaba and Wolaita area (Ayalew, *et al*., 2015) and in Halaba (Wondwosen, *et al*., 2016). After rotation of chickpea soil N increased to 0.24 % N in Wolaita and Halaba. Research reports indicated that a legume crop can increase the yield of the succeeding cereal crop by increasing the availability of soil N (i.e., N effect) (Stevenson and van Kessel, 1996; Garrido and Lopez-Bellido, 2001). High concentrations of soil mineral N can result from the release of mineral N from legume residues incorporated into the soil (Doughton and McKenzie, 1984). Legume residue can contribute more mineral N to the soil through mineralization, as compared to the cereal residue, because legume residue generally had a higher N content and a lower C: N ratio. In this study it was found that soil N increased by 56 % after chickpea crop from the initial level of 0.16 % N. This result is in agreement with the report of Hayat and Ali (2010) where soil N was 42 % higher with mash bean (*Vigna mungo* L.) under P fertilization than non-legume sorghum.

In our chickpea-wheat study, wheat with low rate (39 kg ha^{-1} urea) of fertilization produced a grain yield 19 % over non N fertilized wheat. The yield of cereals grown after legumes is generally increased as much as by 80 % compared with cereals grown after cereals (Hayat and Ali, 2010). Legumes in a cropping system help to maintain soil fertility and leave more moisture in sub-soil because legumes require less water, nutrients and are of short-duration compared to cereals (Badaruddin and Mayer, 1994; Hayat and Ali, 2010). The present data revealed that the increase in grain yield under the current cropping system was possible by using chickpea in rotation and application of low rate of nitrogen.

Research results on wheat in Southern Ethiopia (Wassie and Mamo, 2013) indicated that applying of 50 kg ha^{-1} urea and 100 kg ha^{-1} phosphorus resulted in 5.2 tonnes ha^{-1} biomass and 1.8 tonnes ha^{-1} grain yield, which is comparable with the grain yield obtained from the zero fertilizer chickpea-wheat rotation plot (1.9 tonnes ha^{-1}) in the current study. In another study, wheat following faba bean resulted in a grain yield increase of 3.4 tonnes ha^{-1}, whereas wheat after wheat gave 2.4 tonnes ha^{-1} (Gorfu *et al*., 2000). In the same report, a continuous wheat rotation resulted in relatively shorter wheat plants, reflecting a positive contribution of faba bean as N source for wheat growth. Improvement in cereal yield following mono-cropped legumes

were in the range of 0.5 to 3 tonnes ha^{-1}, representing a 30 to 35 % increase over yield obtained via cereal cropping sequences (Peoples and Craswell, 1992; Wani *et al.*, 1995).

Chickpea increased soil N. However inoculum strains had no effect on N soil content. This result suggests that, selection of an appropriate chickpea cultivar and year-to-year rotation plays an important role in improving soil N content. Bidlack *et al.* (2007) reported a similar observation of the benefit of soil N to wheat, through legume accumulated N in two years.

In most crop growing areas, and in particular in southern Ethiopia, access to affordable fertilizer is limited. Most farmers either apply a low rate of fertilizer or do not apply any at all. The use of high yielding cultivars, however, cannot express their genetic potential because nutrients are the yield limiting factors. Nitrogen is a key factor in crop yield. As a system management tool in our study, we combined the chickpea-wheat rotation with a reduced rate of N fertilizer. Nitrogen use efficiency of the mineral fertilizer application was calculated and the result was found to be low (30.5 %). Such low NUE indicate loss of fertilizer N resulting in reduction in crop yield. Losses of fertilizer N could be caused by leaching or denitrification. Davis *et al*. (2003) reported a NUE of 32.8%, similar to the 33% NUE for world cereal production reported in 1999. Gaseous losses of N from soil systems due to denitrification are influenced by numerous soil properties including soil water content, pH, and temperature (Pu *et al*., 1999) but are largely controlled by the availability of water-soluble or readily decomposable organic matter and the lack of available oxygen (Burford and Bremner, 1975). In aerobic conditions, denitrifying bacteria use oxygen as their terminal electron acceptor. However, when oxygen becomes limited these facultative bacteria are able to use NO_3 or nitrite as an alternative acceptor, thereby releasing N_2 into the atmosphere as biological oxidation of organic matter continues. Such systems are not sustainable and should be avoided in order to maintain agricultural productivity and soil fertility.

In this study, the NUE value of 30.5% indicated the low utilization of available N. This can be risky as more N is lost from soil. This result is evidence for the poor efficiency of the crop and emphasis should be given to select appropriate cultivar. Worldwide, NUE for cereal production is approximately 33%. The unaccounted 67% represents a $15.9 billion annual loss of N fertilizer (Raun and Johnson, 1999). According to Badaruddin and Meyer (1994), the NUE value

in wheat following a legume crop is greater than that of wheat following fallow and of continuous wheat. Garrido and Lopez-Bellido, (2001) indicated that NUE was increased as a result of rotation, wheat yields for monoculture at the maximum N rate (150 kg N ha^{-1}) were lower than those obtained with rotations at the minimum N rate (50 kg N ha^{-1}). N application rates with low NUE values increase risk of nitrogen losses and should be avoided in order to protect the environment. Brentrup and Palliere (2013) evaluated NUE value of 80-90% as a well-balanced input and output at application rates of 144 and 192 kg N ha^{-1}. In this study we can conclude that low NUE indicated N loss and reduced yield.

The move towards sustainable agriculture has encouraged a renewed interest in crop rotations and their effect on N use efficiency. The availability of N immobilized in legume residue for subsequent crops varies widely, depending on a number of factors such as cultivar, rainfall, and soil. Legume N is mineralized more slowly and used more efficiently than high rates of chemical N fertilizer. Rotations trigger changes in soil N sources, which obviously affect N use efficiency. Badaruddin and Meyer (1994), Stockdale *et al*. (1997) and Yamoah *et al*. (1997) have stressed the greater N use efficiency of rotations as compared to monoculture, highlighting the fact that this efficiency is particularly enhanced by legume rotations.

Farmers in southern Ethiopia should consider growing chickpea as an option to increase soil fertility and growing a profitable and nutritious crop. Chickpea grows under residual moisture without competing for land resources with the main-season cereal crops grown in the area. In current practice, legumes are not widely used in crop rotations where it would be possible to reduce the cost of N fertilizer via N_2 fixation. This research also impacts southern Ethiopian situations where famers uproot chickpea at maturity, leaving no biomass in the soil. But through training and demonstration, farmers in the area started to adopt harvesting methods of chickpea like any other cereal crop, to leave the root and some part of the straw. In the search for a more rational farming system, greater importance should be attached to chickpea both as a source of N and as an environmentally friendly crop.

6. Preface

Pulse crop production in Ethiopia is gaining importance and is accounted for 14 percent of cropped land area (Central Statistics Authority of Ethiopia, 2013). However the lack of improved technology coupled with marginal soils has contributed to low yield of chickpea. In order to solve this problem agronomic and breeding strategies should focus on improving management practices such as early seeding dates (Chapter 3) and inoculation of chickpea for nitrogen fixation (Chapter 4 and 5). The inclusion of better management practices, legumes and rhizobia in a cropping system does not always guarantee the attainment of high yield and optimal level of symbiotic nitrogen fixation in the field. Several environmental factors including drought, temperature and soil status are known to negatively affect the symbiosis and nitrogen fixation process and, thus, reduce the actual amount of grain yield and nitrogen fixed by a given legume in the field. For a sustainable productivity the use of genetic variability in tolerance to most environmental stress factors is important.

Development of cropping systems that are able to efficiently use water and nitrogen are essential to maximize yield, reduce production costs and environmental pollution through poor N fertilizer uptake. Research conducted for N fixation over cool and warm season legumes revealed association of drought sensitivity with transport and accumulation of high concentrations of ureides, amino acids used in N transport from root to shoot in warm-season legumes. In the previous chapters the research focused on how various technologies could be used to increase yield of chickpea and the subsequent crops. This chapter investigates the variability of chickpea cultivars in response to drought stress affecting nitrogen fixation. In addition it discusses seed amino acids in relation to moisture stress. The generated information is useful in screening chickpea germplasm for increased N fixation and drought tolerance.

6. Variability of Chickpea Cultivars for Nitrogen Fixation and Seed Composition under Soil Water Deficit

6.1 Introduction

Understanding of the physiological processes during the most stress-sensitive growth stages of chickpea is essential for establishing strategies for crop improvement and management practices to optimize N_2 fixation in cropping systems (Serraj and Adu-Gyamfi, 2004).

Soil moisture plays a critical role in both nodule formation and N_2 fixation (Gan *et al*., 2008). Low soil moisture during the early stages of the plant growth decreases nodule formation, and low moisture during late vegetative to early flowering period decreases efficiency of N_2 fixation (Williams and Mallorca, 1984; Beck *et al*., 1991; Gan *et al*., 2005; Gan *et al*., 2008). An early onset of terminal drought could disrupt symbiotic N_2 fixation which in turn severely impact the seed yield of chickpea (Wery *et al*., 1988).

Inoculation with rhizobium strains resulted in a significant increase in grain yield (van Kessel and Hartley, 2000; Valimohammedi *et al*., 2007; Ibsa, 2013). There are many factors (soil pH, soil moisture, organic matter, native strains, soil temperature etc.) that can prevent nitrogen fixation (Mohammadi *et. al*., 2012). Soil moisture is known as the most important factor that limits nitrogen fixation. Nitrogen fixation activity of some grain legumes including peanut was sensitive to soil drying (Devi *et al*., 2009). Yield improvement may be possible by identifying cultivars with less disruptive nitrogen fixation under water deficit conditions (Devi *et. al*., 2013).

Labidi *et al*. (2009) conducted an experiment to assess relative tolerance of five chickpea genotypes to drought and investigated the relationships between the degrees of sensitivity of plant growth and N content to drought and nodule, leaf and root traits. They found drought limited plant growth of two genotypes and decreased N content in three other genotypes. The other three genotypes had a N shortage associated with an increase in nodule mortality and a restriction of nodule growth. Genotypic differences for water stress effects on nodule, root and leaf traits were limited to (i) a change in the root to shoot ratio (ii) a loss of chlorophylls and (iii) nodule mortality. Each of these traits was considered as an indicator of stress sensitivity.

According to Ashraf and Iram (2005), drought did not influence the colonization of roots by rhizobia; rather it suppressed the growth of nodules. The high sensitivity of chickpea nodule development compared to other plant parts suggested that water deficit specifically affected nodule development during the process of nodulation and early crop N-fixation. Inhibition of nodule development in stressed plants is due to restriction of carbohydrate transport from leaves to nodule (Diaz del and Layzell, 1995; Singh and Singh, 2006). Pena-Cabriales and Castellanos (1993) reported that water stress imposed during vegetative growth compared to reproduction was more detrimental to nodulation and nitrogen fixation. Imposing water deficit conditions for 45 days to 15- day-old well established plants of *Phaesolus vulgaris* (haricot bean) and *Sesbania aculeate* (Daincha) reduced shoot mass and nodule mass of both species, but the reduction was more pronounced in *P. vulgaris* than in *S. aculeate* (Ashraf and Iram 2005).

Research conducted for N fixation over cool and warm season legumes revealed association of drought sensitivity with transport and accumulation of high concentrations of ureides, amino acids used in N transport from root to shoot in warm-season legumes (Peoples *et al*., 1985 and 1987; Sinclair and Serraj, 1995; Herridge and Rose, 2000). These warm season legumes with low or minimal concentrations of ureides had characteristics of increased tolerance to drought (Sinclair and Serraj, 1995). Leaf free amino acids such as alanine, γ- aminobutyric acid, proline, asparagine and glutamine concentrations were higher in water-stressed common bean (*Phaseolus vulgaris* L.) compared with control plants (Raggi, 1994).

In cool season legumes, amide (glutamine or asparagine) transport is the major N transport form. Research in chickpea suggested that drought increased leaf ureide accumulation in drought-sensitive cultivars and decreased total N, alanine and asparagine concentrations over time. Drought-tolerant chickpea cultivars maintained ureide and amide concentrations during drought. Of the chickpea cultivars examined, Myles was the most droughts tolerant and CDC Chico was the least (Thavarajah and Ball, 2006).

Chemical composition and nutrient value of chickpea makes the crop an important food for mankind. Water stressed environmental condition is one of the most limiting factors determining the composition of organic compounds and mineral elements of chickpea. Concentration of mineral elements in plants declines significantly under drought as a consequence of moisture

stress (Kahlil *et al*., 2014). Results indicated that a higher amount of grain protein and soluble sugars were found under moisture stress however starch content decreased. Chemical analysis for mineral composition of Kabuli chickpea showed that accumulation of K and Mg increases and total P, Zn, Ca and Fe decreases in the samples of the moisture stress environment (Chandana and Pratima, 2013). Bueckert *et al*. (2011) found grain nutrient were affected by environment and genotype. Contrary to increased protein and soluble sugars in Kabuli seeds, Nayyar *et al*. (2006); Kahlil *et al*. (2014) reported a significantly greater reduction in the accumulation of phenylalanine , tyrosine, tryptophan, valine, alanine and histidine and minerals (Ca, P, Fe) due to stress in kabuli seed compared with desi seed**.**

The objectives of this study were to evaluate the differences in nitrogen fixation activity and associated seed free amino acid composition of fifteen chickpea cultivars under soil water deficit conditions, and to quantify the plant tissue ureides, alanine, asparagine and total N as indicators of N fixation across the cultivars.

6.2 Materials and Methods

Fifteen chickpea (*C. arietinum* L.) cultivars, selected based on their response to stress, and obtained from different sources (Table 6-1) were used for the experiment.

Table 6-1 List of chickpea cultivars included in the experiment

No.	Variety name	Test code /Pedigree	Type	Source
1	Habru	FLIP-88-42c	Kabuli	Ethiopia
2	Mastewal	ICCV 92006	Desi	Ethiopia
3	Ethiopian landrace	n.a	Desi	Ethiopia
4	Amit	Selection from landrace (Bulgaria)	Kabuli	Canada
5	CDC Chico	G1/C188-220	Kabuli	Canada
6	CDC Corinne	ICC12512-1	Desi	Canada
7	CDC Frontier	FLIP91-22C/ICC14912	Kabuli	Canada
8	CDC Leader	FLIP95-48c/CISN-SP-99 PL 21117	Kabuli	Canada
9	CDC Orion	FLIP95-48c/93-120-63k	Kabuli	Canada
10	CDC 820-32	95NN-12/FLIP 97 133c	Kabuli	Canada
11	ICCV 2	F3[(k850 x GW5/7)xP458]xF3(L550 x Guamuchil)-2	Kabuli	ICARDA
12	ILC 533	Not traced (Egypt)	Kabuli	ICARDA
13	ILC 588	NEC 1628	Kabuli	ICARDA
14	ILC 3182	Not traced (India)	Kabuli	ICARDA
15	ILC 3279	Landrace from former USSR	Kabuli	ICARDA

Field soil from Dutch Growers garden center (Saskatoon) was used for the experiment. A composite sample of the soil was sent to ALS Environmental Saskatoon, Canada for soil texture and property analysis (Table 6-2).

Table 6-2 Soil Properties of the experiment as analyzed by ALS Environmental Saskatoon, Canada

Soil texture	pH	Available Cations (mg/kg)	Available micronutrients (mg/kg)	Available Nitrate (mg/kg)	Available phosphate (mg/kg)
Sandy loam (67% sand, 23% silt and 10% clay)	7.62	Sodium (Na) 75	Copper (Cu) 0.62	126	118
		Potassium (K) 957	Iron (Fe) 29.6		
		Calcium (Ca) 3130	Manganese (Mn) 3.75		
		Magnesium (Mg) 449	Zinc (Zn) 2.57		

Field soil for the experiment was obtained from Dutch growers Ltd.

Eight liter volume plastic pots were placed on the greenhouse bench and filled with 5 kg of air dry field soil (67% sand, 23% silt and 10% clay). Pots were watered and drained to field capacity two days before seeding. The experiment was arranged as a completely randomized design with three replications and repeated twice. Temperature in the greenhouse was maintained at $23/20^0$C day/night with photoperiod 18/6 hours (day/night). Light was supplemented in the night with 1000W high pressure sodium (HPS) light bulb (Sylvania). Within each replication the cultivar was seeded three times per stress treatment and harvest period. The first run of the experiment was conducted from June 6 to October 10, 2014 and the second experiment was conducted from August 10 to December 19, 2014.

The two water deficit treatments were:

Moisture at 70 % of the starting field capacity throughout the growing cycle as the control

Moisture stress at the flowering stage, created by lowering the moisture from 70 % down to 30 % field capacity, and then maintaining 30 % until maturity

To calculate the field capacity, at the beginning of the experiments four pots were filled with a known weight of soil, then saturated with water and allowed to drain freely for a period of 24 hours, until there was no change in weight. The difference between the weight of wet soil and

dry soil was used to calculate 100 % water holding capacity, and was equivalent to all the water the soil could hold between its upper and lower limit, or field capacity to wilting point.

Three seed were seeded per pot and later thinned to one plant per pot at the two leaf stage. A commercial peat based chickpea inoculant (Becker Underwood-Canada) was used for all cultivars at seeding. At emergence, N-free solution with the following macro and micro elements was added to each pot: phosphorus with ca $(H_2PO_4)_2.H_2O$ (18.3 g lit^{-1}), sulfur with K_2SO_4 (36.5 g lit^{-1}), potassium with KCl (25.9 g lit^{-1}), molybdenum with $NaMO_4.2H_2O$ (226.7 mg lit^{-1}), boron with H_3BO_3 (1.3g lit^{-1}), manganese with $MnSO_4.H_2O$ (2.3 g lit^{-1}), zinc with $ZnSO_4.7H_2O$ (2.6 g lit^{-1}) and copper with $CuSO_4.5H_2O$. The macro and micro nutrients stock solution was prepared with 4 liters of water separately and 250 ml of solution was applied to each pot, and adjusted with water for the 70 % field capacity. Watering was done every other day by weighing each pot to adjust the evapotranspiration water loss to 70 and 30 % field capacity.

Each cultivar was seeded in three pots per moisture treatment and per replication. All plants were grown under the 70 % field capacity until each cultivar reached first flowering. After first flowering the soil moisture content was reduced to 30 % field capacity, by withholding the water until the total weight measures 30 % field capacity, for those pots receiving the treatment.

The effect of moisture stress on nitrogen fixation and cultivar differences, stem nitrate concentration and ureide concentration was determined in stems and leaves of 15 chickpea cultivars. Measurements were done per plant basis for agronomic and physiological parameters. Plants were harvested at flowering and 15 days after flowering in both stressed and control treatment to ensure differences in Biological Nitrogen Fixation (BNF) and seed composition. Cultivars were analyzed for nodule number, nodule dry weight, shoot dry weight, 100-seed weight, seed protein concentration, nitrogen yield per plant, stem and leaf ureide concentration, leaf and seed amino acid concentration, and plant total nitrogen, with particular emphasis on identifying relationships between these characteristics with BNF. Results presented are the average of six single plants obtained from two time replicates of the experiment.

6.2.1 Plant Sample Preparation

Plant samples were taken at first flowering and 15 days after first flowering. Plant samples were separated into leaf and stem partitions for drying. Drying of stem and leaf samples were done in an oven at 65^{o}C for 48 hours. Dried petioles and stems were ground using a cyclone mill (1mm particle size) whereas dried leaf samples were ground with a jar-mill shaker using glass beads together with the leaf samples in 5ml micro centrifuge tubes.

6.2.2 Nitrate and Ureide Analysis

Extractions of nitrate from dried ground stem was done following the protocol of Cataldo *et al*. (1975), with 50 mg of sample mixed with 10 ml of hot deionized water in a closed test tube. The mix was kept in a water bath at 80ºC for 30 minutes. After cooling down, to remove chlorophyll from the sample 0.5g of magnesium carbonate plus calcium hydroxide was added to the supernatant, then the mixture was centrifuged at 4500 rpm for 4 minutes. Extracts were stored in the freezer at -20ºC until quantification. For chemical determination of nitrate, a standard stock solution was prepared by dissolving 3.01 g lit^{-1} KNO_3 to prepare standards containing 0-60 ug NO_3-N in a 0.25 ml aliquot. Two reagents were prepared by dissolving 5 g of salicylic acid in 100 ml of 96% sulphuric acid (reagent A), and 40 g of NaOH in 500 ml of deionized water (reagent B). To each 0.25 ml extract or standard, 0.8 ml of reagent A was added to the mix and after 20 minutes at room temperature, 19 ml of reagent B was then added slowly. The mixture formed a yellow color and the absorption was measured at 410 nm with a spectrophotometer.

Ureide concentration was determined according to Young and Conway (1942) with modifications (de Silva *et al*., 1996). Ureides were extracted from 35 mg of dried, ground tissue (Leaves and stem). Samples were homogenized in 1 mL 0.2N sodium hydroxide (NaOH), added to1.5 mL microfuge tubes, boiled at 100°C for 15 minutes, cooled and centrifuged at 13,000 g for 3 min. The supernatant in 1.5 mL microfuge tubes was stored in freezer in upright position until ureide determination. Allantoin was used to prepare standards containing 0 to 300 uM then added 200 ul of 0.5 M NaOH, boiled at 100°C for 15 minutes. Both tissue extract and standard was boiled in 200 ul 0.65M HCL for 4 minutes at 100°C, then cooled. For color quantification 200 ul phosphate buffer (pH 7), 200 ul of freshly prepared phenylhydrazine-C l (0.165 g 50 ml^{-1}) for 5 minutes, and cooled on ice for 5 minutes. Color was developed with the addition of 1 ml of

concentrated HCl and 200 ul of freshly prepared potassium ferricyanide (KFeCN). Ureide concentration was determined spectrophotometrically against an allantoin standard at 522 nm within 15 min of addition of KFeCN. Ureide and nitrate concentrations are presented in μmol g^{-1} dry weight. Plant Nitrogen content was measured by combustion (LECO CNS 2000, St. Joseph, MI). Tissue N concentration was presented as percentage tissue dry weight (%). Dried grain samples were taken from each treatment and grounded using a ball mill, reserved for ^{15} N natural abundance plant samples at the University of Saskatchewan. From each experimental unit, approximately 2 mg of ground seed sample was weighed into a tin capsule (8×5 mm). The capsule was then closed, compressed and placed in 96-well micro plates. Samples were analyzed for natural abundance and isotopic diluted composition using a 20-20 Mass Spectrometer interfaced with an ANCA-GSL sample converter (Europa Scientific, Crewe, UK).Nitrogen derived from the atmosphere was estimated as described in chapter 4. The calculation of % Ndfa was done without a specific B-value. Wheat was used as reference crop to calculate % Ndfa. Peoples *et al.* (1995a) suggested when the % Ndfa is < 50 % and δ^{15}N is not small the impact of the B-value is less.

6.2.3 Free Amino Acid Extraction and Analysis

Ground leaf and seed samples were used to analyze physiologically free amino acids (Mustafa *et al*., 2007), using a gas chromatograph method (Phenomenex liner P/N AGO-4680, EZ : faast, California, USA). Ground samples (100mg) were extracted with 50 % ethanol at 50^0C on an orbital shaker for 20 minutes. Following centrifugation (10,000 rpm for 15 minutes), 0.5 ml of an aliquot is taken and mixed with 100 uL of internal standard in a glass preparation vial. Mixtures of amino acids standards were used to quantify amino acid concentrations. Norvalin was used as the internal standard and quantification were carried out by comparing sample peak areas to the standard's peak areas. Solid phase extraction is performed via a sorbent packed tip, attached to a 1.5 ml syringe that binds amino acids while allowing interfering compounds to flow through. Amino acids on sorbent were then extruded into the sample vial and quickly derivatized with reagent at room temperature in aqueous solution. Derivatized amino acids migrate to the organic layer for additional separation from interfering compounds. An aliquot from the organic layer was analyzed by GC with FID detector. The GC is setup with, injection 1:15 @ 250^0C, 2 uL;

hydrogen carrier 1.5ml min^{-1}; oven program 32^0C min^{-1} from 110^0 to 320^0 (6.56 minute run), and detector temperature of 320^0C.

6.2.4 Statistical analysis

Data were subjected to analysis of variance using SAS system for Windows version 9.2 (SAS Institute, 2009). Both cultivars and moisture treatments were considered as fixed. Significantly different treatments ($p<0.01$) were identified with Tukey's method ($P<0.05$) in Proc Mixed. Means and SE were generated using least square means, and LSD was calculated with the means statement.

6.3 Results

Analysis of variance revealed a highly significant (P<0.01) effect of cultivar by treatment interaction for stem ureide and nitrate concentration. Cultivar had a significant effect (P<0.05) for nodule number, nodule dry weight, 100-seed weight, seed protein concentration, leaf ureide concentration, and percent nitrogen derived from the atmosphere (% Ndfa). Moisture stress had a significant effect on all parameters under study except leaf ureide concentration. Seed protein concentration significantly increased under moisture stress condition (Table 6-3 and 6-4).

Above ground plant biomass (g plant^{-1}) was significantly reduced by moisture stress in most cultivars except Amit, CDC 820-32 and CDC Frontier.

Stem ureide concentration was influenced by moisture stress indicating that nitrogen fixation was disrupted due to low moisture. Except for CDC Chico and Habru, the concentration of ureide declined in all cultivars when moisture was lowered to 30 percent field capacity after flowering (Fig.6-1).

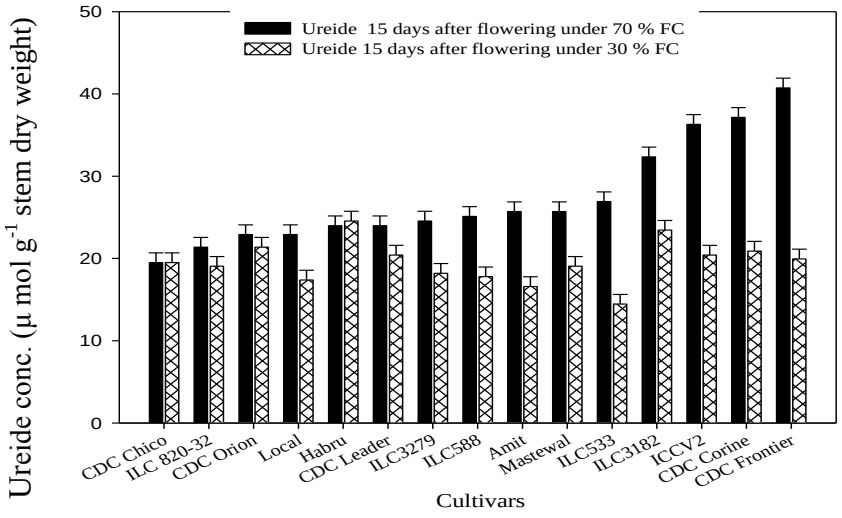

Figure 6.1 Stem ureide concentrations (µ mol g^{-1} stem dry weight) of 15 chickpea cultivars after 15 days of flowering under 70 and 30 % field capacity (FC). LSD is 1.22 µ mol ureide g^{-1} stem dry weight.

Table 6-3 Analysis of variance (mean and p-values) for total biomass, nodule number, seed weight, leaf and stem ureide concentrations, stem nitrate concentrations, and grain qualities of fifteen chickpea cultivars tested under moisture stress

Treatment	Above ground biomass (g Plant^{-1})	Nodule number Plant^{-1}	Nodule dry weight g Plant^{-1}	Shoot dry weight (mg Plant^{-1})	Seed weight plant^{-1} (g)	100 Seed Weight (g)	Stem Ureide (µ mol g^{-1})	Stem nitrate (µ mol g^{-1})	Leaf ureide (µ mol g^{-1})	Seed protein (%)	Protein yield (g plant^{-1})	%Ndfa (Seed)
Cultivars(C)	0.002	0.038	0.011	0.001	0.001	0.001	0.001	0.038	0.007	0.001	0.001	0.25
Moisture treatment (T)	0.001	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.727	0.008	0.001	0.001
C * T	0.019	0.749	0.433	0.961	0.173	0.721	0.003	0.001	0.412	0.436	0.129	0.003
Mean	4.28	13.5	1.53	10.2	3.96	24.1	22.9	9.62	53.4	25.2	0.948	22.1
SE	0.122	1.08	0.01	0.387	0.19	0.619	1.02	1.06	1.04	0.225	0.039	0.49

Nitrate concentration in stem tissue of chickpea cultivars Habru, CDC Orion, CDC Leader, Amit, Mastewal, ILC 3279 and ILC 533 increased significantly under stress condition, whereas its concentrations in Ethiopian landrace, CDC Chico, CDC Corinne and CDC 820-32 were not affected by the stress occurred after first flowering (Fig 6-2).

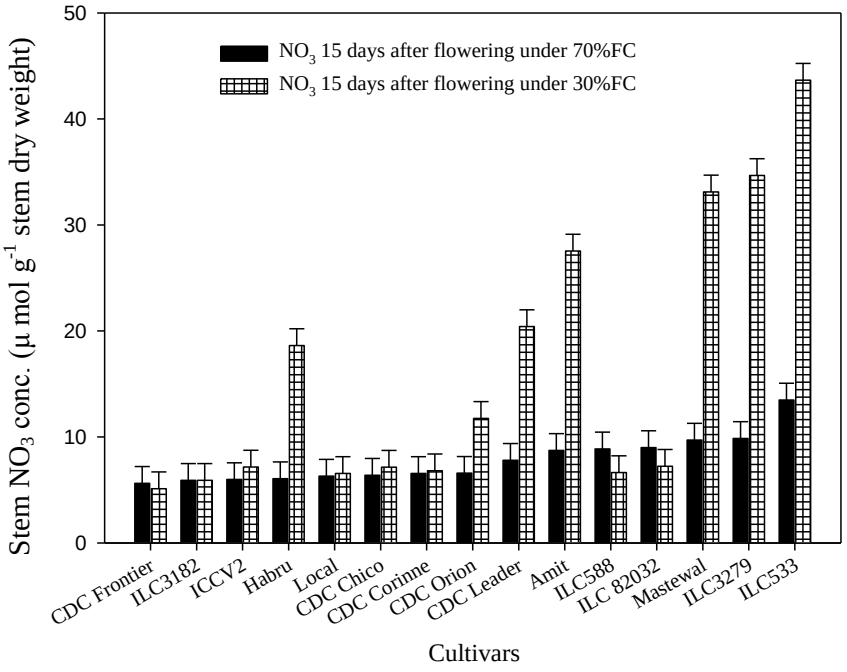

Figure 6-2 Stem NO_3 concentration (µ mol g^{-1} stem dry weight) of 15 chickpea cultivars after 15 days of flowering under 70 and 30 % field capacity (FC). LSD is 2.43 µ mol nitrate g^{-1} stem dry weight.

Nitrate concentration was also measured at different growth stages under optimum and low moisture conditions. Nitrate content varied among cultivars under optimum conditions at first flowering stage, indicating that cultivars varied in their efficiency to absorb N from the soil and accumulate it into their tissue. But nitrate content declined 15 days after flowering, and only some cultivars increased their nitrate content under low moisture conditions (Fig.6-3).

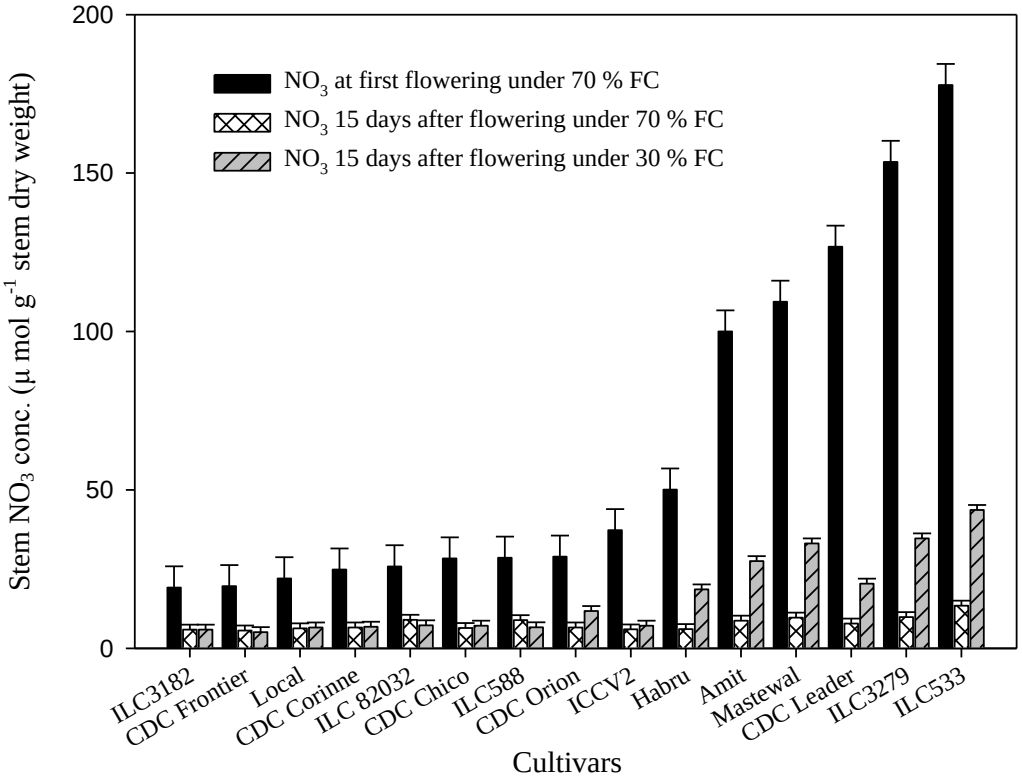

Figure 6-3 Stem NO_3 concentrations (µ mol g^{-1} dry weight) of 15 chickpea cultivars at different growth stages and moisture stress levels. LSD is 13.4 µ mol g^{-1} stem dry weights at first flowering and 2.43 µ mol g^{-1} stem dry weight 15 days after flowering.

Both leaf and stem ureide were also compared at same growth stage. Leaf ureide content was greater than stem ureide, even under optimum water conditions (Fig 6-3).

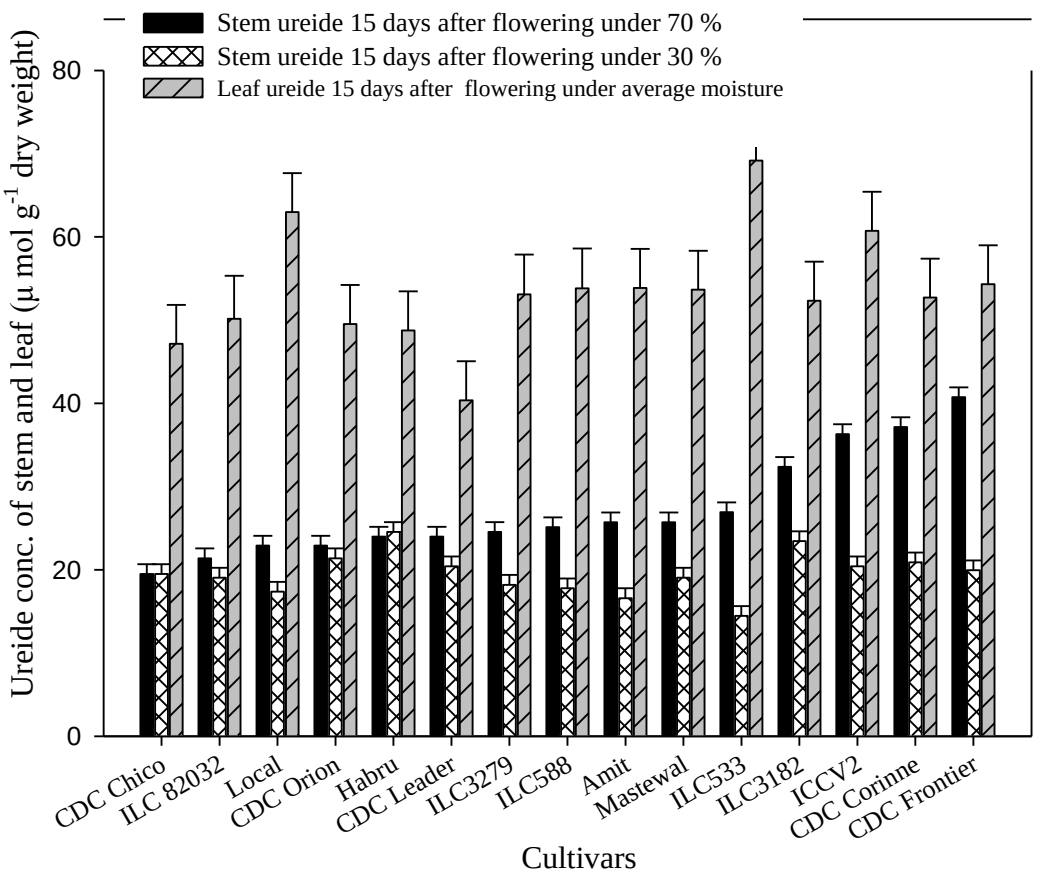

Figure 6-4 Stem (at 70 and 30 % FC) and leaf ureide concentration (µ mol g^{-1} dry weight) of 15 chickpea cultivars. LSD is 1.22 µ mol ureide g^{-1} stem dry weight and 10.2 µ mol ureide g^{-1} leaf dry weight

Cultivars varied in their leaf ureide concentration 15 days after flowering (Fig. 6-5). Moisture stress had no significant effect on leaf ureide concentration (Fig. 6-5). Leaf ureide concentration varied from 40-67 µ mol g^{-1} dry leaf samples. CDC Leader had the lowest concentration, whereas ILC 533 and Ethiopian landrace had the highest leaf ureide concentration (Fig.6-5).

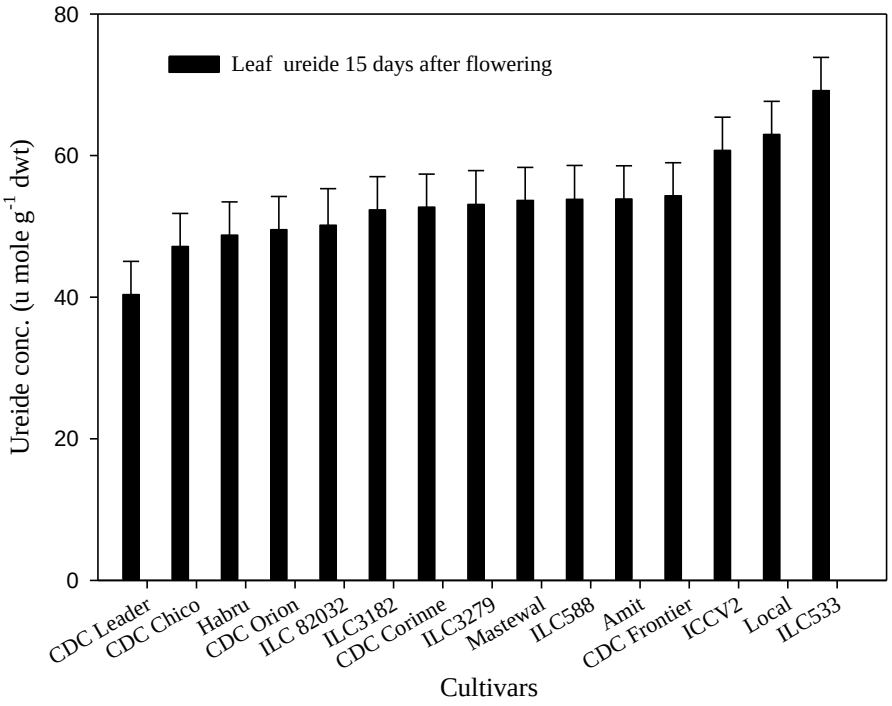

Figure 6-5 Leaf ureide concentration (μ mol g^{-1} dry weight) of 15 chickpea cultivars 15 days after first flowering across moisture treatments. LSD is 10.2 μ mol ureide g^{-1} leaf dry weight

Chickpea cultivars had no significant variation in their percentage nitrogen derived from the atmosphere (% Ndfa), which ranged from 19.9 % (ILC 588) to 26.4 % (Amit). The analysis revealed a highly significant ($P<0.001$) interaction of cultivar by moisture on % Ndfa (Figure 6-6). Moisture stress (30 % FC) reduced % Ndfa in cultivars Amit, CDC Corinne, CDC Frontier, ILC 3182, and ILC 3279. Under moisture stress % Ndfa in ILC 533 was 28.7 compared to 18.3 under control (70% FC), this might be due to more leaf ureide concentration. At optimum moisture (70 % FC), % Ndfa was 24.5 but stress reduced the % Ndfa to 19.6. Moisture stress (30 % FC) increased seed protein concentration (25.6 %) as compared to 24.8 % at 70% FC. Whereas the total protein yield per plant was greater under optimum moisture because yield was greater. Cultivars showed significant variation for grain protein concentration (Fig. 6-7) but the interaction of cultivar x moisture was not significant ($p< 0.05$) for grain protein.

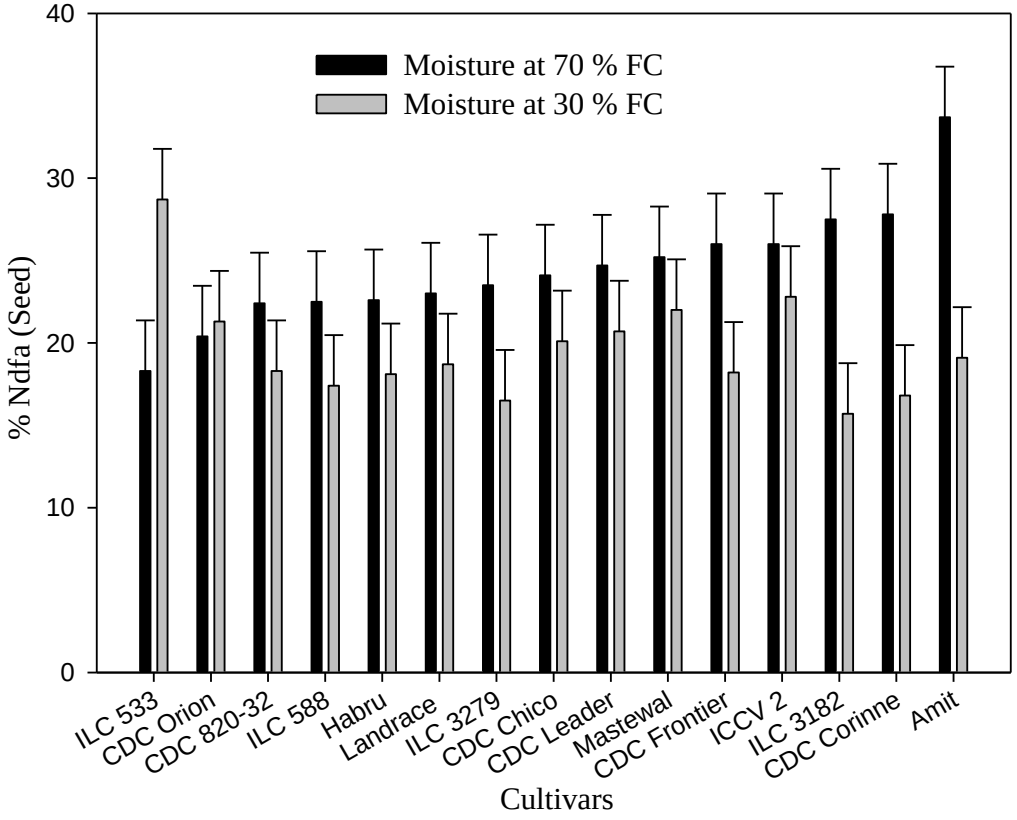

Figure 6-6 Cultivar x moisture effect on % Ndfa of 15 chickpea cultivars 15 days after first flowering under moisture treatments. LSD is 8.4

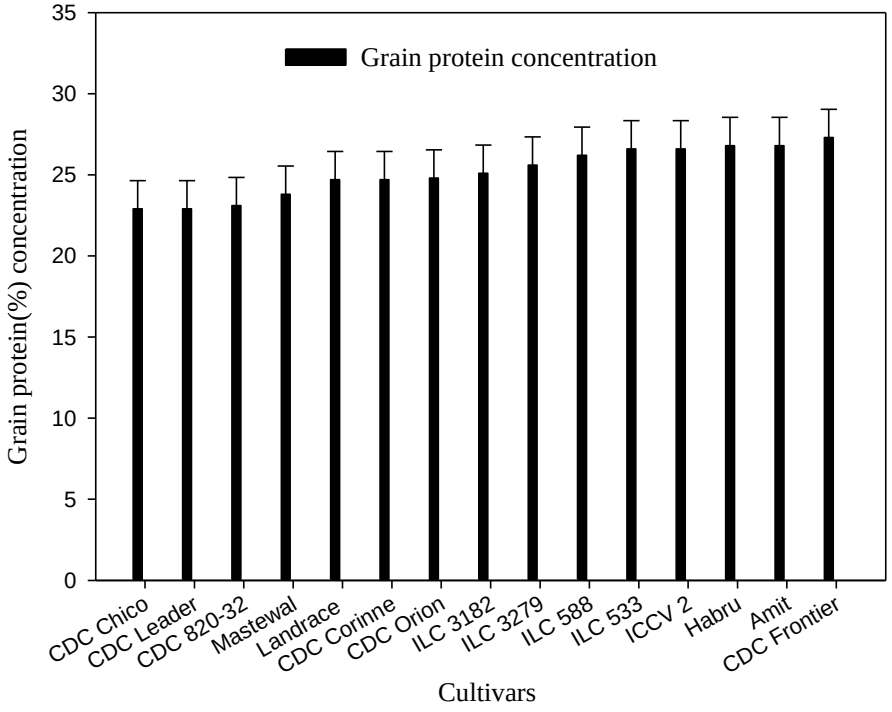

Figure 6-7 Grain protein (%) concentration of 15 chickpea cultivars across moisture treatments. LSD is 1.69

Leaf free amino acid differences, 15 days after first flowering, were found to vary among chickpea cultivars and moisture treatments. Moisture stress (30 % field capacity) reduced valine, leucine, threonine, methionine, lysine, histidine, proline and asparagine concentrations. Significant cultivar differences in leaf free amino acid concentrations were measured in μ mol g^{-1} leaf dry weight (Table 6-4), ranging for valine (0.022-0.1), leucine (0.063-0.205), threonine (0.244-1.22), methionine (0.013-0.043), lysine (0.252-0.808), histidine (0.027-0.105), tryptophan (0.005-0.014), serine (15.1-32.6) and proline (2.99-10.9).

Among cultivars ILC 533 exhibited greater concentration of valine, leucine, threonine, lysine, methionine, histidine and tryptophan, whereas ILC 588 measured the lowest concentration of valine and CDC leader had low concentrations of threonine, lysine, methionine, histidine and tryptophan (Table 6-4).

Table 6-4 Mean leaf free amino acid (μ mol g^{-1} leaf dry weight) for each cultivar, 15 days after first flowering, across moisture treatments (70 % field capacity (FC), 30 % field capacity (FC)) at temperature of 23/20°C day/night

	Leaf free amino acids (μ mol g^{-1} dry sample)								
	Valine	Leucine	Isoleucine	Threonine	Methionine	Phenyl-alanine	Lysine	Histidine	Tryptophan
Habru	0.034^{cd}	0.126^{bcd}	0.118	0.515^{cd}	0.018^{cd}	0.010	0.423^{cde}	0.035^{cd}	0.005^{c}
Mastewal	0.04^{bcd}	0.205^{a}	0.149	1.02^{abc}	0.04^{ab}	0.009	0.665^{abc}	0.105^{a}	0.009^{abc}
Landrace	0.059^{bcd}	0.188^{a}	0.093	0.857^{abc}	0.028^{abcd}	0.010	0.549^{abcd}	0.068^{abcd}	0.008^{abc}
Amit	0.057^{bcd}	0.158^{abc}	0.105	0.853^{abc}	0.018^{cd}	0.013	0.458^{cde}	0.04^{bcd}	0.005^{c}
CDC Chico	0.062^{abcd}	0.193^{a}	0.15	1.12^{ab}	0.031^{abcd}	0.009	0.586^{abcd}	0.08^{ab}	0.008^{abc}
CDC Corinne	0.058^{bcd}	0.171^{ab}	0.139	0.83^{abc}	0.034^{abc}	0.009	0.519^{bcd}	0.048^{bcd}	0.006^{bc}
CDC Leader	0.031^{cd}	0.063^{e}	0.166	0.244^{d}	0.013^{d}	0.012	0.252^{e}	0.027^{d}	0.005^{c}
CDC Frontier	0.063^{abc}	0.151^{abcd}	0.131	0.929^{abc}	0.032^{abcd}	0.009	0.529^{bcd}	0.053^{bcd}	0.012^{ab}
CDC Orion	0.031^{cd}	0.101^{de}	0.157	0.497^{cd}	0.023^{bcd}	0.009	0.504^{bcde}	0.041^{bcd}	0.006^{bc}
CDC 820-32	0.079^{ab}	0.102^{de}	0.117	0.891^{abc}	0.043^{a}	0.009	0.592^{abcd}	0.067^{abcd}	0.009^{abc}
ILC 3182	0.065^{abc}	0.104^{cde}	0.145	0.672^{abcd}	0.027^{abcd}	0.009	0.391^{de}	0.027^{d}	0.005^{c}
ILC 3279	0.057^{bcd}	0.132^{bcd}	0.175	0.737^{abcd}	0.03^{abcd}	0.011	0.774^{ab}	0.08^{ab}	0.013^{a}
ILC 533	0.1^{a}	0.203^{a}	0.102	1.22^{a}	0.043^{a}	0.012	0.808^{a}	0.103^{a}	0.014^{a}
ILC 588	0.022^{d}	0.108^{cde}	0.208	0.599^{bcd}	0.024^{abcd}	0.009	0.56^{abcd}	0.066^{abcd}	0.009^{abc}
ICCV 2	0.051^{bcd}	0.203^{a}	0.124	1.05^{abc}	0.026^{abcd}	0.008	0.639^{abcd}	0.076^{abc}	0.009^{abc}
Mean	0.054	0.147	0.138	0.803	0.029	0.010	0.548	0.061	0.008
SE	0.016	0.019	0.04	0.272	0.011	0.003	0.093	0.016	0.002
LSD	0.041	0.054	0.092	0.555	0.020	0.005	0.262	0.042	0.006
Control (70%FC)	0.067^{a}	0.183^{a}	0.138	1.04^{a}	0.038^{a}	0.010	0.659^{a}	0.078^{a}	0.009
Stress (30%FC)	0.041^{b}	0.112^{b}	0.139	0.56^{b}	0.019^{b}	0.010	0.441^{b}	0.044^{b}	0.007
SE	0.009	0.007	0.026	0.2	0.008	0.002	0.034	0.008	0.001
LSD	0.015	0.019	0.034	0.203	0.007	0.002	0.096	0.015	0.002

Table 6-4 Mean leaf free amino acid (µ mol g-1 dry sample) for each cultivar, 15 days after first flowering, across moisture treatments (70 % field capacity (FC), 30 % field capacity (FC)) at temperature of 23/20°C day/night (continued)

	Leaf free amino acids (µ mol g^{-1} dry sample)								
	Alanine	Glycine	Serine	Proline	Asparagine	Aspartate	Hydroxy -proline	Glutam- ic acid	Glutam- ine
Habru	1.05	0.158	21.8^{bcde}	4.97^{cd}	0.031	0.013	0.136	0.021	0.032
Mastewal	1.21	0.03	26.2^{abc}	8.59^{ab}	0.038	0.014	0.201	0.042	0.063
Landrace	1.38	0.069	15.1^{e}	7.53^{bc}	0.038	0.112	0.182	0.037	0.051
Amit	1.07	0.128	15.9^{e}	5.34^{cd}	0.028	0.015	0.185	0.024	0.063
CDC Chico	1.11	0.015	19.4^{cde}	7.33^{bc}	0.036	0.015	0.193	0.035	0.053
CDC Corinne	1.12	0.068	17.1^{de}	6.66^{bc}	0.034	0.016	0.253	0.03	0.041
CDC Leader	1.13	0.121	32.6^{a}	2.99^{d}	0.024	0.019	0.455	0.077	0.032
CDC Frontier	1.05	0.149	21.2^{bcde}	7.24^{bc}	0.039	0.015	0.166	0.022	0.066
CDC Orion	1.24	0.066	25.3^{abcd}	5.27^{cd}	0.037	0.007	0.061	0.031	0.044
ILC 82032	1.27	0.087	29.4^{ab}	6.88^{bc}	0.043	0.031	0.318	0.071	0.066
ILC 3182	1.26	0.173	26.8^{abc}	5.47^{bcd}	0.042	0.015	0.697	0.034	0.046
ILC 3279	1.19	0.088	25.5^{abcd}	7.83^{abc}	0.035	0.011	0.253	0.289	0.175
ILC 533	1.28	0.081	21.2^{bcde}	10.9^{a}	0.052	0.018	0.178	0.044	0.075
ILC 588	1.31	0.104	27.7^{abc}	7.2^{bc}	0.036	0.022	0.22	0.049	0.052
ICCV 2	1.2	0.055	26.0^{abcd}	7.26^{bc}	0.036	0.012	0.096	0.035	0.063
Mean	1.19	0.093	23.4	6.77	0.037	0.016	0.239	0.056	0.062
SE	0.12	0.042	3.71	1.28	0.011	0.008	0.187	0.068	0.037
LSD	0.326	0.115	9.08	3.16	0.021	0.015	0.447	0.192	0.1
Control (70%FC)	1.23	0.099	22.2	8.38^{a}	0.042^{a}	0.016	0.207	0.072	0.059
Stress (30%FC)	1.16	0.087	24.6	5.15^{b}	0.032^{b}	0.015	0.272	0.039	0.064
SE	0.052	0.018	2.18	0.743	0.009	0.006	0.114	0.026	0.016
LSD	0.119	0.042	3.32	1.15	0.007	0.005	0.163	0.07	0.036

Table 6-5 Mean and p-values of free amino acids in seed of fifteen chickpea cultivars tested under combined moisture treatment

	Alanine	Glycine	Valine	Leucine	Isole-ucine	Threo-nine	Serine	Proline	Aspartic acid	Meth-ionine	Glutamic acid	Pheny-lalanine
Cultivars(G)	0.001	0.001	0.001	0.001	0.003	0.001	0.002	0.001	0.002	0.001	0.001	0.001
Moisture treatment (T)	0.001	0.001	0.046	0.005	0.001	0.002	0.181	0.001	0.001	0.005	0.003	0.003
G * T	0.001	0.001	0.001	0.001	0.002	0.001	0.006	0.001	0.072	0.076	0.001	0.001
Mean	0.167	0.106	0.074	0.029	0.028	0.087	0.277	0.142	0.034	0.035	0.062	0.041
SE	0.02	0.009	0.007	0.003	0.002	0.017	0.029	0.008	0.004	0.002	0.006	0.003

Stress (30 % field capacity) significantly reduced leaf amino acids valine, leucine, methionine, lysine histidine, threonine, asparagine and proline. Regardless of the moisture treatment, cultivars also significantly varied for valine, leucine, threonine, methionine, lysine histidine, tryptophan, serine and proline (Table 6-4).

A significant interaction of cultivars by moisture stress was observed for most amino acids in the seed except for aspartic acid and methionine, indicating that cultivars responded differently to stress with regard to seed amino acids. In general, moisture stress reduced seed amino acid in most cultivars (Tables 6-5). CDC Corinne and ILC 3182 had greater concentrations of valine, leucine, isoleucine, threonine, serine, alanine and glycine under optimum moisture treatment, whereas CDC Chico had greater concentrations of valine, leucine, threonine, serine, and alanine under moisture stress treatment (Table 6-5). Seed alanine ranged from 0.02 (Landrace) to 1.18 (CDC Corinne) μ mol g^{-1} sample. Glycine ranged 0.039 to 0.573 for Ethiopian landrace and CDC Corinne, respectively. Proline concentration was high for CDC Chico (0.389 μ mol g^{-1}) and low (0.05 μ mol g^{-1}) for CDC 820-32 (Table 6-6).

Leaf amino acids were associated with seed weight, total nitrogen and grain nitrogen under moisture stress. Mean leaf concentrations of proline, threonine and serine were greater than the rest of amino acids (6.4). Proline was negatively associated ($r = -0.63$) with seed weight (g $plant^{-1}$) under stress ($P< 0.05$). A positive association ($r= 0.4$, $P>0.05$) of proline was observed with plant total N and grain N ($r= 0.6$, $p<0.05$) under moisture stress (Fig. 6-8).

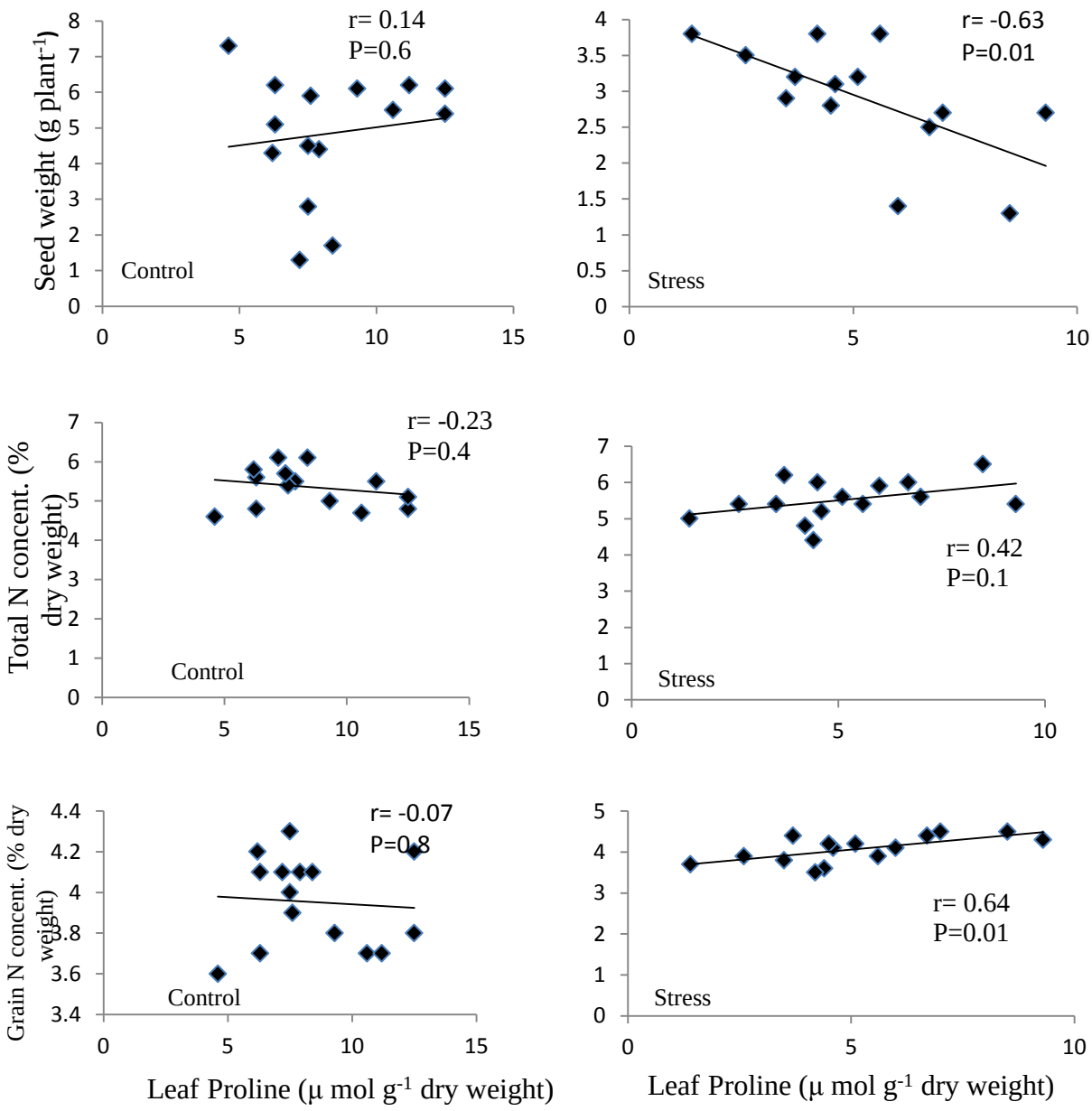

Figure 6-8 Association of leaf proline with seed weight (g plant^{-1}), total plant N (% dry weight) and grain N (% dry weight) over 15 chickpea cultivars under control (70 % FC) and stress (30 % FC) moisture ($p<0.05$)

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Leaf threonine was negatively associated (r = -0.66, p<0.05) with seed weight (g plant^{-1}) under stress. A positive association (r= 0.46, P> 0.05) of threonine was observed with plant total N and grain N (r= 0.59, p< 0.05) under moisture stress respectively (Fig. 6.9).

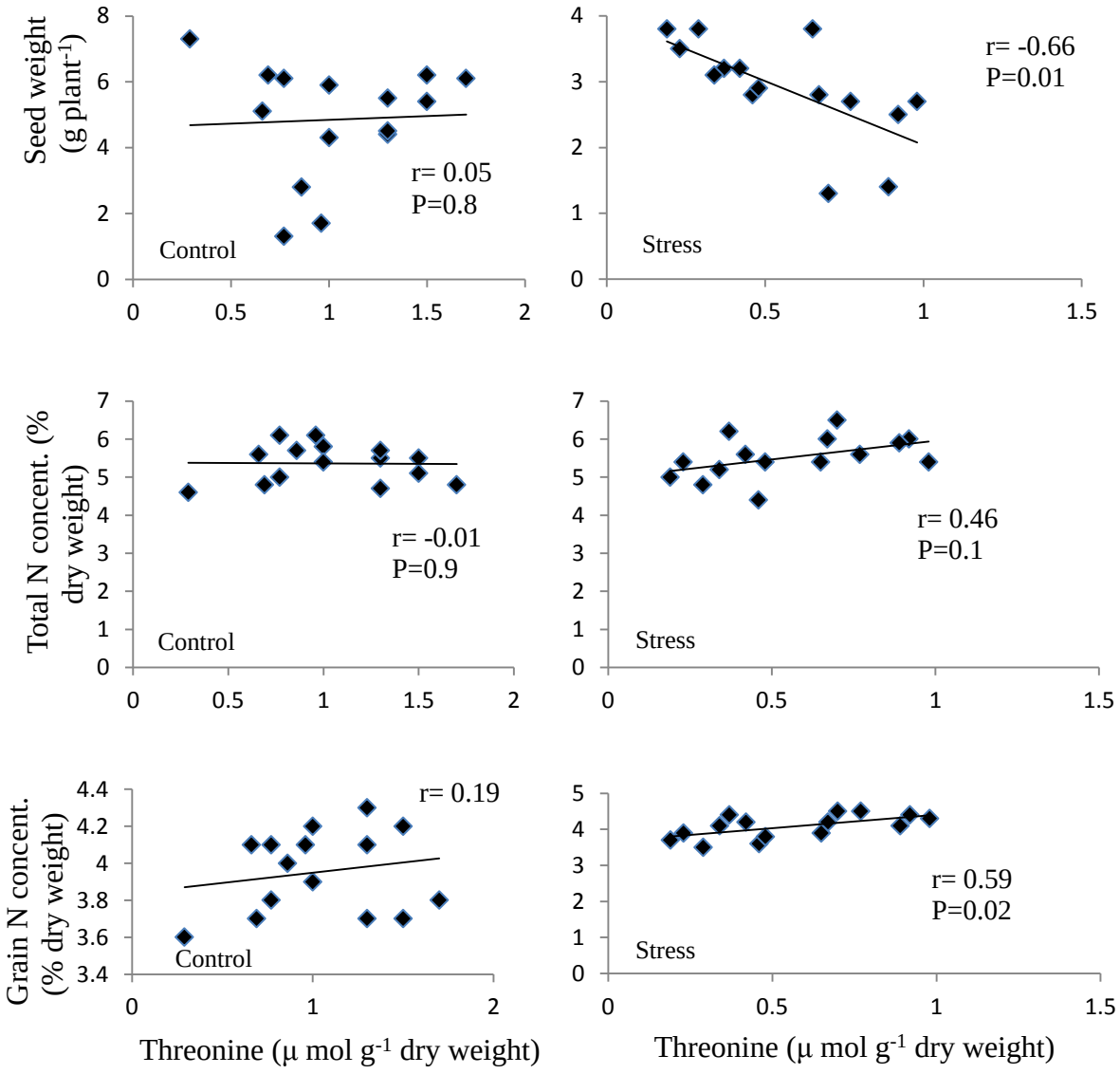

Figure 6-9 Association of leaf threonine with seed weight (g plant^{-1}), total plant N (% dry weight) and grain N (% dry weight) over 15 chickpea cultivars under control (70 % FC) and stress (30 % FC) moisture (p<0.05)

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Table 6-6 Mean free amino acid (µ mol g-1 dry seed weight) in seed under 70 % (control) and 30 % (stress) field capacity among fifteen chickpea cultivars

	Seed free amino acids (µ mol g^{-1} dry seed weight)													
	Valine		Leucine		Isoleucine		Threonine		Serine		Methionine		Phenylalanine	
Cultivars	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Habru	0.028	0.092*	0.018	0.034	0.029	0.022	0.012	0.117*	0.095	0.259	0.026	0.026	0.155*	0.025
Mastewal	0.011	0.04	0.011	0.01	0.019	0.019	0.015	0.004	0.088	0.116	0.02	0.034	0.063*	0.016
Landrace	0.017	0.033	0.008	0.011	0.035*	0.01	0.007	0.021	0.083	0.118	0.087	0.027	0.076*	0.029
Amit	0.041	0.099	0.014	0.036	0.036*	0.008	0.018	0.107	0.102	0.206	0.023	0.025	0.043	0.051
CDC Chico	0.052	0.125*	0.014	0.05*	0.076*	0.019	0.039	0.155*	0.365	0.794*	0.067	0.043	0.056	0.043
CDC Corinne	0.357*	0.087	0.164*	0.029	0.062*	0.012	0.562*	0.094	0.834*	0.163	0.036	0.018	0.013	0.044
CDC Leader	0.04	0.028	0.017	0.01	0.032	0.012	0.026	0.023	0.258	0.119	0.019	0.017	0.037	0.048
CDC Frontier	0.005	0.079*	0.007	0.026	0.051*	0.01	0.018	0.056	0.138	0.165	0.033	0.026	0.056*	0.018
CDC Orion	0.069	0.039	0.019	0.016	0.049*	0.022	0.085	0.045	0.268	0.329	0.061	0.042	0.06*	0.022
CDC 820-32	0.16*	0.072	0.032	0.037	0.031	0.01	0.188*	0.074	0.209	0.152	0.039	0.025	0.029	0.023
ILC 3182	0.212*	0.109	0.102*	0.029	0.051*	0.01	0.332*	0.025	0.462	0.627	0.049	0.02	0.014	0.034
ILC 3279	0.058	0.044	0.025	0.017	0.044*	0.012	0.048	0.023	0.814*	0.206	0.039	0.022	0.037	0.037
ILC 533	0.028	0.041	0.013	0.018	0.067*	0.017	0.017	0.039	0.393	0.165	0.048	0.029	0.029	0.021
ILC 588	0.067	0.028	0.042*	0.012	0.034	0.015	0.132*	0.017	0.375	0.065	0.04	0.031	0.033	0.031
ICCV 2	0.079	0.066	0.028	0.025	0.008	0.018	0.053	0.137	0.138	0.165	0.025	0.034	0.041	0.039
Mean	0.074		0.029		0.028		0.087		0.277		0.035		0.041	
Std. Error	0.007		0.003		0.002		0.012		0.029		0.002		0.003	
LSD	0.06		0.027		0.023		0.099		0.37		0.027		0.035	

* Significant at $p<0.05$

Table 6-6 Mean free amino acid (μ mol g^{-1} dry seed weight) in seed under 70 % (control) and 30 % (stress) field capacity among fifteen chickpea cultivars (continued).

Cultivars	Free amino acids (μ mol g^{-1} dry seed weight) in seed									
	Alanine		Glycine		Proline		Aspartic acid		Glutamic acid	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Habru	0.04	0.193	0.062	0.11	0.051	0.163*	0.032	0.034	0.345*	0.03
Mastewal	0.093	0.062	0.068	0.059	0.205	0.209	0.005	0.048	0.075	0.044
Landrace	0.022	0.041	0.057	0.039	0.065	0.107	0.021	0.117	0.198*	0.044
Amit	0.065	0.181	0.083	0.103	0.117	0.233*	0.028	0.141	0.043	0.073
CDC Chico	0.101	0.277*	0.097	0.142	0.389*	0.231	0.005	0.018	0.055	0.06
CDC Corinne	1.18*	0.143	0.573*	0.097	0.187	0.123	0.04	0.043	0.021	0.053
CDC Leader	0.121	0.018	0.086	0.043	0.115	0.078	0.009	0.061	0.04	0.075
CDC Frontier	0.043	0.209*	0.065	0.073	0.124	0.113	0.039	0.061	0.057	0.024
CDC Orion	0.1	0.086	0.128	0.063	0.09	0.092	0.009	0.034	0.071	0.043
CDC 820-32	0.219	0.211	0.145	0.118	0.086	0.055	0.008	0.036	0.042	0.043
ILC 3182	0.383*	0.161	0.235*	0.083	0.167*	0.073	0.018	0.077	0.023	0.05
ILC 3279	0.101	0.115	0.073	0.061	0.196*	0.096	0.005	0.041	0.037	0.045
ILC 533	0.029	0.103	0.056	0.058	0.279*	0.113	0.007	0.028	0.041	0.032
ILC 588	0.365*	0.034	0.126*	0.041	0.191*	0.064	0.016	0.02	0.043	0.041
ICCV 2	0.128	0.123	0.124	0.094	0.095	0.143	0.006	0.024	0.039	0.039
Mean		0.167		0.106		0.142		0.034		0.062
Std. Error		0.022		0.009		0.008		0.004		0.006
LSD		0.161		0.067		0.072		0.05		0.059

6.4 Discussion and Conclusions

Ureide concentration varied by tissue (Fig. 6-1 and 6-5). Variability in ureide concentration in response to moisture stress have been reported (Serraj *et al*., 1999b; Purcell *et al.*, 2000; Purcell and Specht, 2004; Thavarajah and Ball, 2006; Kabahuma, 2013; Coleto *et al*., 2014). The average leaf ureide concentration was two or more times greater than the concentration in stems. Moisture stress reduced stem ureide concentration by 37 % in dry stem sample. ILC 533 at 70 % field capacity (FC) had 27 μ mol g^{-1} dry weight stem ureide concentration, but under stress (30 % FC) ureide concentration was reduced to 14 μ mol g^{-1} dry weights. However, highest leaf ureide concentration was observed for ILC 533 (69 μ mol g^{-1} dry weight). This might be due to fast translocation of ureide from stem to leaf, and decreased catabolism of leaf ureide. The highest concentration (74 μ mol g^{-1} dry weight) of leaf uriede recorded at 30 % FC from ILC 533 supports this idea. Coleto *et al*. (2014); reported increased levels of ureides in stems and leaves of common bean during the drought treatment. Contrary to the current finding Kabahuma (2013), observed a higher concentration of ureide in stem than leaves of common beans under stress.

Stem ureide under stress was similar to the control treatment in CDC Chico, CDC Orion and Habru. This might indicate reduced catabolism of stem ureide in those cultivars. Under more severe water-deficit conditions, there were greater and more consistent increases in petiole ureide concentration for Jackson (drought tolerant) and Biloxi (sensitive). Jackson, however, had lower petiole ureide concentration than Biloxi throughout the measurement period for both well-watered and water-deficit treatments. Petiole ureide accumulation may result from decreased ureide catabolism (Purcell *et al.*, 1998).

Ranking for stem and leaf ureide also varied with chickpea cultivars. For stems, at 70 % FC the top five cultivars ranked in order: CDC Frontier ≥ CDC Corinne ≥ ICCV2 ≥ ILC 3182 ≥ ILC 533. Similaly, for stem ureide at 30 % FC, ILC 533 had the lowest ureide concentration. Ureide concentration ranking in leaves was ILC 533 ≥ Landrace ≥ ICCV2 ≥ CDC Frontier ≥ Amit across moisture treatments.

Cultivars varied for stem and leaf ureide concentration across moisture treatment for example ILC 533 had high stem and leaf ureide concentration under optimum moisture and low stem concentration of ureide under stress, similarly, significant interaction effect of cultivar by

moisture reduced % Ndfa in Amit, CDC Corinne, CDC Frontier, ILC 3182, and ILC 3279 under stress (30%) condition. On the other hand stress increased % Ndfa (28.7) in ILC 533(Fig. 6-6). Leaf ureide concentration was the highest in ILC 533 under stress (Fig. 6-5). This indicated ureide concentration might have effect on N_2 fixation. Similarly, King and Purcell (2005), reported ureides and total free amino acids in leaves and nodules increased during water deficits and coincided with a decline in N_2 fixation. Fixation of N_2 recovered to 74% to 90% of control levels 2 days after re-watering drought-stressed plants, but leaf ureides and total nodule amino acids remained elevated in cultivar KS4895. Asparagine accounted for 82% of the increase in nodule amino acids relative to well-watered plants at 2 days after re-watering. These results indicate that leaf ureides and nodule asparagine do not feedback inhibit N_2 fixation. Compounds whose increase and decrease in concentration mirrored the decline and recovery of N_2 fixation included nodule ureides, nodule aspartate, and several amino acids in leaves, indicating that these are potential candidate molecules for feedback inhibition of N_2 fixation.

The pathways of assimilation of N derived from fixation and from soil are different: ammonia derived from symbiotic fixation is converted into ureides, allantoin and allantonic acid, in the nodule and then transported to the shoot in the chemical form in the transpiration stream; in contrast, N taken up from soil, which is primarily nitrate is transported either directly as nitrate or is assimilated into the amino acids asparagine or glutamine in the root prior to transport (Herridge and People, 1990). In this experiment the amount of stem nitrate was measured in order to see the proportion of N products when N fixation is inhibited due to moisture stress. Cultivar difference for nitrate concentration was highly significant and affected by moisture treatments. Moisture stress at 30 % FC increased overall stem nitrate by 62 % compared to the control (70 % FC). Cultivar ILC 533 under stress had the maximum nitrate concentration (24.3 μ mol g^{-1} dry weight) followed by ILC 3279. The lowest nitrate concentration was found on CDC Frontier at 5.4 μ mol g^{-1} dry weight. Interaction of cultivar by moisture treatment showed highly significant ($P< 0.001$) differences for nitrate concentration. A high nitrate concentration at 30 % FC moisture was found in ILC 533, ILC 3279, Mastewal, Amit, CDC Leader and Habru. Cultivars ILC 3182 and CDC Frontier which had highest stem ureides, also had the lowest nitrate under both water levels. In contrast ILC 533, which had a low concentration of stem ureide, exhibited highest nitrate concentration.

The accumulation of some free amino acid in leaves under moisture stress was similar with optimum water conditions (Table 6-4). Greater concentration of serine, proline and alanine compared to other free amino acids was observed. Such increase of free amino acids concentration might have delayed wilting of stressed chickpea cultivars. Research results indicate that a stressful environment results in an overproduction of proline and other free amino acids in plants which in turn impart stress tolerance by maintaining cell turgor or osmotic balance (Shamsul, *et al*., 2012). However, reports have shown accumulation of other free amino acids under stress conditions e.g., aspartic acid, glutamic acid and glutamine in cotton; asparagine, aspartic acid, serine and glycine in maize; ornithine, arginine and glutamic acid in detached rice leaves; and serine, proline, alanine and asparagine in peas and chickpeas (Slukhai and Shvedova, 1972; Hanower and Brzozowska, 1975; Thakur and Rai, 1982; Yang *et al*., 2000; Tavarajah, 2005).

Cultivars differed significantly in some free amino acids accumulations. ILC 533 recorded the maximum amino acid content for proline, valine, leucine, threonine, methionine, lysine, histidine, and tryptophan followed by CDC Chico. The higher accumulation of these osmolytes in ILC 533 was associated with low stem uriede under stress, and could be considered as a strategy to mitigate the effect of moisture stress through rapid translocation of N products. Silvente *et al.* (2012), observed variability in free amino acid measurement in soybean due to stress. The contents of alanine and glutamine decreased whereas aspartate levels increased in leaves. In contrast, they observed no significant differences in the amino acid concentration in the nodules of both cultivars under control and stress conditions. Researchers reported, an increase in free amino acid due to moisture stress in chickpea and concluded that chickpea genotypes possess osmoregulation as a mechanism for moisture stress tolerance (Ashraf and Iram, 2005; Pankaj and Deshmukh , 2008). The free amino acids contributed to the osmolyte pool. Reduced amino acid concentration observed in some cultivars could be due to susceptibility to 30 % FC for 15 days. The result indicated that leaf amino acid measurement could be appropriate to screen for drought tolerant germplasm.

Result presented in Table 6.6 show the interaction effect of cultivar by moisture treatment on free amino acid concentration in seed of chickpea cultivars. The maximum free amino acid concentrations for most cultivars were recorded under optimum moisture. Non- significant

responses to stress were observed for aspartic acid and methionine. Drought stress increased amino acid concentration in seed of CDC Chico. Similar results were found in cowpea plants exposed to drought stress during the flowering stage which led to an increase in the free amino acids concentrations in cowpea and in bean seed (Osman, 2015), in soybean (King and Purcell, 2005) but Gyori *et al.* (1998) did not observe response of soybean for drought. Increased free amino acids concentration like proline in leaves may have positive effects in osmoregulation, as seen in ILC 533 and CDC Chico. Proline concentration was found high under optimum moisture in ILC 533 but other amino acids were not affected by moisture treatment.

Water stress at flowering decreased the total plant biomass and seed weight per plant by 29 % and 47 % respectively (Appendix 6). The effect on the weight of individual seeds was only a 13 % reduction as a result of stress. The deleterious effect of water stress on yield was mainly due to a decrease in pod number (61 %), this would be a result of increased pod abortion. This result is in agreement with the report by Behboudian *et al*. (2001). Moisture stress improved the seed's nutritive value in terms of higher accumulation of protein but harvested total protein yield was reduced by stress.

There are diverse research reports on the influence of moisture on grain yield, nitrogen fixation and agronomic yield attributes, but less information is available on seed composition as affected by moisture stress in chickpea. Seed composition like amino acids and protein are directly influenced by N fixation and assimilation, which in turn is affected by moisture stress. In this experiment the seed composition in chickpea was affected by moisture stress through increasing the protein and some amino acid concentrations.

Amino acid concentrations in leaves (Table 6-4) showed, in general, lower values under stress. The concentration of the following amino acids was affected by the stress: valine, leucine, threonine, methionine, lysine, histidine, asparagine, and proline. Concentrations of, isoleucine, phenylalanine, tryptophan, alanine, glycine, serine, aspartic, hydroxproline, glutamic acid, and glutamine remained same with control treatment. The positive association of proline and threonine with total N and grain N might indicate the contribution of these metabolites in reducing wilting and used as source of N under stress. A decrease in the levels or close to control

values, of threonine (Thr), Glutamate, Glycine, Aspartate, and GABA were observed in *Medicago truncatula* leaves under stress (Cirousse *et al*., 1996; Gil-Quintana *et al*., 2012).

7. General Discussion and Future Research

Chickpea is a potentially profitable pulse crop option for the southern Ethiopian cropping region. It contributes to profitability of farming systems through its ability to fix nitrogen, serves as a source of high protein in the diet, generates income and provides weed and disease breaks for main-season cereal crops. The two major constraints to chickpea production in Ethiopia are terminal drought stress and a proper management package such as the availability of high yielding cultivars, appropriate seeding time, and rhizobium inoculant (Abate *et al.*, 2011).

In southern Ethiopia, due to high population pressure, the land holding per household is less than a hectare. This results in frequent cereal cultivation on the land without fallowing. Pulse crops like chickpea help to improve the fertility of the soil because farmers cannot afford inorganic fertilizer to grow cereals. In addition, chickpea helps to reduce malnutrition and improves human health, especially for the poor who cannot afford animal protein. Chickpea is an excellent source of protein, fiber, complex carbohydrates, vitamins, and minerals (Menale *et al*., 2009).

Low yield of chickpea is mainly due to lack of improved technology. A report indicated, more than 75 % of the chickpea production in the area used local landraces and as a result a productivity gap of 1.2 tonnes ha^{-1} was observed (International Food Policy Research Institute, 2010). In southern Ethiopia, less attention has been given to chickpea research and farmers have no access to improved cultivars. Crop rotation is not a common practice by farmers in these areas. In addition, low fertilizer or no fertilizer input is common in the region. Therefore, my thesis objectives were to evaluate the agronomic performance of chickpea cultivars grown under different agro-ecology including seeding date, rhizobium inoculation and residual nitrogen effect on wheat grain yield. In addition, the thesis also examined how soil water deficit impacted nitrogen fixation and seed composition among chickpea cultivars.

Seeding date can be used as a strategy to avoid high temperatures during flowering, and to reduce the effect of water deficit. The optimal time to seed chickpea will depend on the interaction between the location and the available cultivar. Yield response of cultivars varies with different seeding times and their agronomic characteristics. This was demonstrated for the cultivars Habru and Ejere, when they were seeded at a mid or late seeding date they tended to flower and mature early in all locations. This is an indication of stress resulting in a short

growing period through shortening the vegetative phase, and flowering coinciding with temperatures more conducive to subsequent pod development. In an experiment with four seeding periods, late seeding times in chickpea resulted in a reduction of yield, plant height, pods plant^{-1} and 100-seed weight whereas yield obtained from the first two early seeding dates was statistically similar and not affected by stress (Kumar *et al*., 2003; Kumar *et al*., 2008).

Seeding date affected different agronomic characteristics. Yield attributes such as number of pods plant^{-1}, 100-seed weight, grain and straw yield were significantly affected by the date of seeding in northern India (Tiwari and Meena, 2014). Number of pods plant^{-1}was considered as the most important yield determinant varied significantly under different dates of seeding. Ray *et al*. (2011) also found that early seeding results better in terms of seed yield, number of pods plant^{-1}, seed pod^{-1} and test weight. Early seeding date, September 3 to 10, provides favorable environmental conditions for the plant growth and development and is associated with more growth attributes that result in higher yield. Similar results and explanations were given by Prasad *et al*. (2012) in India, when planting was delayed from December 1 to December 20, significant yield reductions ensued. Kabir *et al.* (2009) also reported in a study of three chickpea cultivars and five seeding dates in Bangladesh, as seeding was delayed from November 22 to December 22 or January 1, a significant yield reduction occurred.

The seeding date experiment showed that grain yield and 100-seed weight variation was dependent on seeding date and cultivars. Growing chickpea under residual moisture after the main crop harvest should help farmers obtain a double crop and a double harvest. Moreover, the N_2 fixing potential of chickpea can be considered a solution to help resource poor farmers in the area reduce the cost of fertilizer for growing cereal crops following chickpea.

Legume N_2 fixation is not only a function of processes occurring at a microbiological level but is also influenced by factors that impact on plant productivity. Such factors include the environmental (soil moisture and temperature) conditions under which the legume is produced, farmer management practices (seeding time, and inoculant) and soil nitrate status (Peoples *et al*., 1995b; Whitbread *et al*., 2000). Due to the complexity of issues influencing nodulation and effective N_2 fixation, simply including legumes in farming systems is not a guarantee of enhancement of soil N fertility.

Despite the potential of chickpea to provide economic and rotational benefits, there are factors that limit nodulation, N fixation and yield. Chickpea grain yield is associated with nodulation, low nodulation result in low N_2 fixation and low yield (Kyei-Boahen *et al*., 2002; Ben *et al*., 2008). Chickpea nitrogen fixation could be affected if the rhizobium inoculant is ineffective McConnell, *et al*. (2001); there is high concentration of soil nitrate, soil moisture stress and competition with indigenous soil microbial population. The % Ndfa of chickpea is maximised when there is a low level of available soil nitrate. The inhibitory effects of soil nitrate on nodulation of pulses, and chickpea in particular, is well documented (Doughton *et al*., 1993; Herridge *et al*., 1998), because the highest levels of N_2 fixation are typically achieved in situations where low levels of seeding soil nitrate, i.e. <25 kg N ha^{-1} existed.

The field experiment to study the response of cultivars to *Rhizobium* inoculation confirmed that environmental factors and host symbiont compatibility can dramatically affect the nitrogen fixation process. Despite low total soil nitrogen concentration, and the very small population (< 10 μg g^{-1}) of native rhizobia (Ibsa, 2013) at Wolaita, seed yield did not respond to inoculation in 2011 and 2012. The explanation for this could be that the commercial strain of chickpea inoculant used in this study was poorly adapted to this soil-climatic region, evident from the small nodule number and nodules being less efficient. This result is in agreement with the findings of McConnell, *et al*. (2001) where they reported no significant differences in shoot N detected in chickpea between inoculated and uninoculated chickpea prior to anthesis. Failure of inoculant to adapt to the environment results in poor nodulation, and hence, poor N fixation (Elias *et al*., 2004; Deaker *et al*., 2004; Elias, 2009).

Results from the inoculation experiment indicated cultivars vary for % Ndfa regardless of inoculation treatment. The % Ndfa ranged from 26% to 54% although variation was higher across environments. There is strong evidence that chickpea is capable of meeting its N requirements for high grain yields by utilizing a combination of N_2 fixation and soil mineral N (Doughton *et al.*, 1993). There is less certainty, however, regarding the ability of chickpea to contribute N to the farming system and to sustain soil N fertility. As discussed earlier, to maintain soil N fertility in cereal-legume rotations, fixed N_2 needs to exceed N partitioned to seed or vegetative matter that is removed from the system (Peoples *et al*., 1995b). Therefore, to

confirm if residual nitrogen from chickpea could benefit the succeeding wheat crop in the following cropping calendar, a chickpea- wheat rotation experiment was conducted.

Soil sample analyzed from previously chickpea seeded plot indicated that total soil N concentration increased from 0.16 % N to 0.2% N which is a 56 % increase from the initial 0.16 % N. The explanation for increased N availability in the soil could be due to cultivar N fixation coupled with the release of N from legume residue incorporated into the soil. This result is similar with a 42 % soil N increase reported by Hayat and Ali (2010).

The rotational value of legume crops including chickpea is, however, more commonly quantified by increases in soil mineral N (N benefit) and the biomass and grain yields of subsequent cereal crops (rotational benefits). Much of the N fixed by grain legumes is removed at harvest; the remainder becomes available to subsequent crops following mineralization, may be incorporated into the soil organic matter, or as with fertilizer N, may be lost from the cropping system (Marcellos *et al*. 1998; Khan *et al*.2003; van Kessel and Hartley, 2000). The rotational benefit of chickpea was evaluated by combining management practices like application of low N fertilizer rate on wheat. The yield difference from the non-fertilized chickpea wheat plot was 19 % yield increase in fertilized plots. In the current study the low NUE indicated much of the soil N was lost from the system. Similar results were reported on low NUE of 34 % at a rate of 112 kg N ha^{-1} yr^{-1} (Johnson and Raun, 2006). Low NUE is a result of the soil-plant system leaking N and the leakage occurring in direct proportion to the degree to which mineral N is present in excess (Raun and Johnson, 1999). Greater NUE can be obtained by increased yields from early maturing and efficient cultivars in their N uptake. Fast growing plants have root systems that more effectively exploit available soil resources (Burns, 1980). Crop health, insect and weed management, moisture and temperature regimes, supplies of nutrients other than N, and use of the best adapted wheat cultivar all contribute to more efficient uptake of available N and greater conversion of plant N to grain yield.

In general farmers in southern Ethiopia can benefit by growing chickpea to increase soil fertility, a benefit aside from growing a profitable and nutritious crop. Chickpea is grown under residual moisture without competing for land resources from the main season cereal crops grown in the area. In current practice legumes are not widely used in crop rotations, and those few farmers seeding chickpea usually remove the total biomass at harvest. Because research was conducted

on farmers' fields, farmers were advised to harvest chickpea for just grain instead of their traditional uprooting practice for grain and stover. Through such management practices, it is possible to reduce the cost of N fertilizer by improving soil fertility through both N_2 fixation and residue incorporation.

As explained earlier in each chapter of this thesis, late seeding exposed cultivars to terminal drought stress. Drought also affected nodule development and hence N fixation (chapter 4 and 5). The field research on inoculation identified poor % Ndfa due to poor nodulation and ineffective nodules. This report corroborates with previous studies of McInnes and Thies (2001), and Elias *et al.* (2004), in which newly evolved strains varied in effectiveness, but with the majority of strains substantially less effective than the original inoculant strain.

Nitrogen fixation rapidly declines under water deficit conditions (Serraj *et al*., 1999a; Purcell *et al*., 2004). Nitrogen fixation is more sensitive to water stress in ureidic (warm-season) legumes than in amidic (cool-season) legumes (Sinclair and Serraj, 1995; Serraj *et al*., 1999a). This (warm-season) includes some species such as cowpea and pigeon pea, which have the reputation of being generally tolerant to drought in terms of plant survival. Moreover, shoot ureide accumulation is observed in drought-stressed plants and it was proposed as one of the causes of nitrogen fixation inhibition (Serraj *et al*., 1999b; King and Purcell, 2005). The correlation between ureide levels and nitrogen fixation inhibition in sensitive and tolerant soybean cultivars was recently corroborated. In the sensitive cultivar ('Biloxi'), N_2 fixation inhibition occurred earlier and was more dramatic than in the tolerant cultivar ('Jackson'). The carbon flux to bacteroids was also more affected in 'Biloxi' than in 'Jackson', due to an earlier inhibition of sucrose synthase activity and a larger decrease of malate concentration in the former. Drought provoked ureide accumulation in nodules of both cultivars, but this accumulation was higher and occurred earlier in 'Biloxi'. However, at this early stage of drought, there was no accumulation of ureides in the leaves of either cultivar. Moreover their research result indicated that a combination of both reduced carbon flux and nitrogen accumulation in nodules, but not in shoots, is involved in the inhibition of N_2 fixation in soybean under early drought (Ladrera *et al*., 2007).

Genetic improvement of legumes for increased tolerance of N fixation to water deficits requires the identification of variability in this trait among potential parental cultivars. The existence of

variations among cultivars within legume species in N_2-fixation sensitivity to water deficit (Serraj *et al*., 1997; Serraj and Sinclair, 1997) indicates that the tolerance trait found in some genotypes may be useful in breeding programmes for effective N_2 fixation under drought conditions in legumes.

Our results confirmed that variability exists among chickpea cultivars for response to water deficit. Cultivars varied in their accumulation of shoot ureide and nitrate. Variation in shoot ureide concentration is in agreement with published findings in different pulse crops (Serraj, *et al*., 1999a; Thavarajah *et al*., 2006; Kabahuma, 2013; Coleto *et al*., 2014). Leaf ureide concentration was two or more times greater than the concentration in stems indicating fast translocation of assimilate towards the sink due to increased stress. Moisture stress reduced stem ureide concentration by 37 % and increased stem nitrate by 62 % compared to the control. Although moisture stress significantly affected and reduced stem ureide compared to the non-stress control treatment, non-significant effect was observed on cultivars CDC Chico, CDC Leader, CDC Orion, CDC 820-32, Landrace and Habru.

Nitrogen metabolism in plant tissues has received great attention under water stress conditions mainly through its relation to protein and amino acid metabolism. Eighteen different amino acid concentrations were estimated in chickpea leaves in my thesis. Moisture stress in general reduced Valine, Leucine, Threonine, Methionine, Histidine, Lysine, Proline, and Asparagine. The higher accumulation of these osmolytes associated with low stem uriede under stress could be considered as a mechanism to minimize the negative effect of drought through rapid translocation of N products. Silvente *et al.* (2012) observed variability in free amino acid measurement in soybean due to stress.

High proline, threonine and serine in leaves may have positive effects in osmoregulation, as seen in ILC 533 and CDC Chico. King and Purcell (2005) showed that total leaf amino acids accumulate under water limitation, with Asparagin showing the largest single increase. Asparagin has been implicated as a potential candidate for feedback inhibition of N_2 fixation (Vadez *et al*., 2000; Todd and Polacco, 2004) and can directly inhibit ureide catabolism (Lukaszewski *et al*., 1992), providing a possible mechanism for ureide accumulation. Asparagin and other nitrogenous compounds may then move through the phloem to play a role, directly or indirectly, in the feedback inhibition of N_2 fixation (Todd *et al*., 2006).

Similarly twelve free amino acids were estimated in seed of 15 chickpea cultivars grown under water deficit. An increase in free amino acids occurred for most cultivars under optimum moisture. A non- significant response to stress treatment was observed for aspartic acid and methionine. Drought stress increased amino acid concentrations in CDC Chico. Osman (2015) reported that plants exposed to drought stress during the flowering stage to lead to an increase in the free amino acid content in cowpea, and in soybean (King and Purcell, 2005).

Improvement of legumes for increased tolerance of N fixation to water deficit could be based on variability in nitrogen fixation capacity, and this research confirms that potential among genotypes. The dependence on shoot ureide as a screening mechanism for chickpea may not be an efficient strategy though. Therefore, screening germplasm for increased tolerance of N fixation to water stress should consider evaluating the root nodule and shoot ureide relationship during the stress period. A research report by Alamillo *et al*. (2010) on the effect of water stress on ureide content in root, stem and leaf parts of plants relying on N fixation as the sole N source revealed that ureide concentration was higher in drought-stressed tissues compared to controls. Increases in ureides in non-nodulated plants were similar to that in stressed tissues from plants cultured under nitrogen fixing conditions. As expected, ureide levels were higher in control, well-watered, tissues from nodulated plants than in the non-nodulated ones. However, water deficit induced a marked increase in the level of ureides even in the absence of nodules.

The results of this study suggest that increasing chickpea productivity on smallholder farms is possible and that the use of improved cultivars with an appropriate agronomic practice, namely early seeding can achieve this goal. However, chickpea seeding time in southern Ethiopia depends on the harvest time of the preceding crop, therefore future research and extension activity should focus on agronomic practices and identify maturity groups of the main-season crops seeded by farmers. For a future research suggestion, Rhizobium inoculant research should test more strains (domestic or imported) to ascertain the best host-strain combination. The influence of water deficit on nitrogen fixation in chickpea indicated that cultivars varied in their accumulation of shoot ureide and nitrate. However, screening germplasm for increased tolerance of N fixation to water stress should consider evaluating leaf amino acid such as serine, proline, and threonine. In addition, emphasis should be given to root nodule and shoot ureide relationships, and root and stem nitrate concentrations during the stress period.

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APPENDIX *1*- Monthly rainfall and mean temperature data for the experimental location of Wolaita for the years 2000-2012.

Year	Janu-ary	Feb-ruary	March	April	May	June	July	August	Septe-mber	Octo-ber	Nove-mber	December
						Rainfall (mm)						
2000	0.6	0.0	31	152	225	198	124	223	170	168	59.8	11.3
2001	19.1	11.6	107	89.8	295	164	234	252	113	140	43.8	42.3
2002	47.8	15.5	122	97.7	173	66.9	90.5	198	54.2	58.6	0.4	96.4
2003	99.5	23.4	62	131	66.6	187	198	263	36.2	62.4	40.3	86
2004	55.4	52.5	41	278	106	103	185	159	63.6	94	143	27.7
2005	8.2	30.7	213	208	323	108	242	NA	152	107	114	5
2006	4.4	25	125	222	143	102	137	257	62.1	133	37.6	121
2007	74.1	35.1	90.9	186	225	259	226	245	249	23.2	15	0
2008	6.1	15.1	10.9	86.7	137	135	225	242	191	207	132	0
2009	13.5	21.3	29.2	129	125	60.6	95.4	122	99.9	146	45.5	164
2010	16.2	97.1	145	189	281	199	113	97.4	131	34.2	18.7	19.8
2011	**13**	**20**	**43**	**63**	**372**	**171**	**158**	**173**	**61**	**18**	**112**	**3**
2012	**0**	**0**	**45**	**181**	**72**	**172**	**197**	**170**	**135**	**13**	**33**	**0**
						Temperature (T^0c)						
2000	21.2	22.7	23.3	21.2	19.7	18.5	17.9	17.9	18.8	19.2	19.8	20.1
2001	19.7	21.7	21	20.6	19.6	18.3	17.7	18.2	18.8	19.5	19.9	21
2002	20.5	21.8	20.8	20.8	19.8	19	19	18.6	19.5	20.2	21.2	20.8
2003	19.9	22.1	22.5	NA	NA	19	17.8	18.1	19.5	20.7	21.3	20
2004	20.7	21.4	22	20.1	20.1	18.5	18	18.5	19	19.3	23.6	21
2005	20.8	22.8	21.8	21.2	19.1	18.9	17.9	17.9	18.8	19.4	20.2	NA
2006	NA	NA	NA	NA	NA	NA	NA	18.3	19.2	19.9	20.3	20.1
2007	20.3	21.3	22.5	20.1	19.5	18.6	18.1	18.1	18.5	19.2	20	20
2008	20.9	20.8	21.4	NA	20.3	19	18	18.5	19.3	19.4	19.6	20.2
2009	20.9	22.4	23.3	21	20.3	19.8	18.7	19.3	19.9	19.9	20.9	20.2
2010	20.3	21.2	21	20.7	20.1	19.4	18.3	18.6	19.4	20.5	21.1	20.4
2011	**20**	**21**	**22**	**22**	**20**	**18**	**18**	**18**	**19**	**20**	**19**	**19**
2012	**20**	**23**	**22**	**19**	**20**	**18**	**17**	**18**	**18**	**20**	**20**	**20**

Source: Southern Nations, Nationality's Peoples Region (SNNPR) meteorological center. NA= Data Not Available

APPENDIX 2- Monthly rainfall and mean temperature data for the experimental location of Halaba for the years 2000-2012.

Year	January	February	March	April	May	June	July	August	September	October	November	December
						Rainfall (mm)						
2000	0.0	0.0	9.0	181	141	72.8	133	65.1	104	107	43.4	23.5
2001	8.5	12.5	190	89.9	98.6	118	122	125	129	47.8	0.0	2.8
2002	29.4	61.8	81.1	86.3	57.2	78.1	71.1	109	87.9	10.9	0.0	123
2003	42.3	62.1	111	140	75.5	73.5	66.8	169	99.5	9.5	48.8	44.0
2004	150	22.3	39.4	184	76.3	34.5	106	112	178	75.2	14.4	0.0
2005	39.0	7.7	65.7	236	84.3	56.1	94.9	70.9	116	37.2	48.2	0.0
2006	4.9	40.6	137	122	50.0	114	135	91.7	59.5	80.4	11.5	27.5
2007	18.6	93.7	85.1	110	161	115	118	120	167	63.3	0.0	0.0
2008	0.0	1.0	26.6	55.0	111	124	107	139	162	74.1	148	2.3
2009	33.1	35.1	47.9	83.4	78.7	83.4	78.3	128	57.9	111	10.3	103
2010	29.4	93.8	132	193	177	53.5	140	99.1	131	32.2	12.8	11.9
2011	**9**	**21**	**37**	**48**	**105**	**121**	**138**	**113**	**49**	**0**	**7**	**0**
2012	**0**	**0**	**36**	**48**	**5**	**42**	**161**	**85**	**63**	**4**	**7**	**0**
						Temperature (T^0c)						
2000	21.1	21.8	22.6	22.6	20.4	19.4	19.1	19.2	19.3	20.0	20.4	20.4
2001	20.8	22.1	21.0	21.5	20.6	19.8	19.2	19.7	19.4	20.2	20.3	21.0
2002	21.4	21.9	21.9	21.8	21.6	20.5	20.4	19.9	20.5	20.6	21.6	21.8
2003	20.8	22.6	22.5	21.7	22.5	20.6	19.4	19.8	NA	NA	NA	NA
2004	NA	21.7	22.6	21.4	21.1	20.1	19.1	19.5	20.1	19.9	20.7	21.3
2005	21.1	22.8	22.7	22.1	20.7	20.3	19.2	20.0	19.9	20.3	19.9	19.9
2006	21.9	22.7	21.9	20.9	20.9	20.1	19.2	19.4	20.4	21.4	20.9	21.9
2007	22.3	22.4	22.2	21.4	21.5	19.6	19.5	19.7	20.2	19.7	20.7	20.4
2008	22.2	22.3	22.6	22.5	21.4	20.0	19.1	19.8	20.6	20.6	19.9	20.6
2009	21.4	22.3	23.7	22.4	22.1	21.5	20.0	20.5	21.5	20.9	21.1	22.1
2010	21.6	22.4	22.0	22.3	22.1	20.9	20.0	20.2	20.7	21.3	21.3	21.5
2011	**22**	**23**	**23**	**23**	**22**	**20**	**20**	**20**	**21**	**21**	**22**	**21**
2012	**22**	**22**	**23**	**22**	**23**	**21**	**20**	**20**	**21**	**21**	**22**	**21**

Source: Southern Nations, Nationality's Peoples Region (SNNPR) meteorological center. NA= Data Not Available

APPENDIX 3 Monthly rainfall and mean temperature data for the experimental location of Butajira for the years 2000-2012.

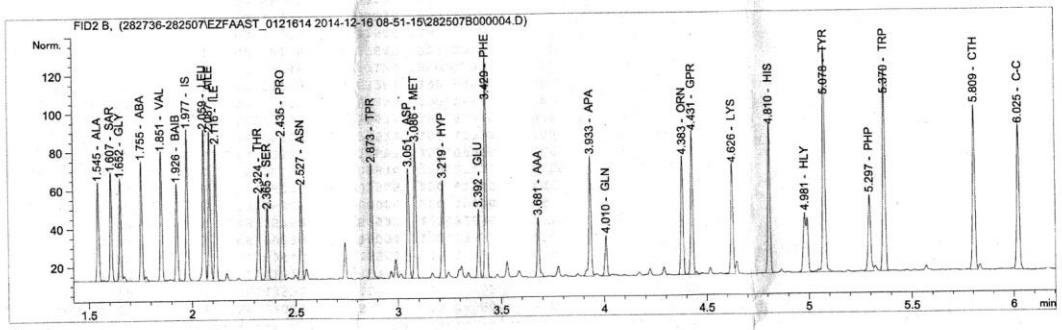
Year	January	February	March	April	May	June	July	August	September	October	November	December
						Rainfall (mm)						
2000	0.0	0.0	6.1	122	75.4	57.8	149	133	55.5	57.0	90.0	102
2001	0.0	59.0	263	59.2	195	234	137	189	174	0.0	0.0	0.0
2002	49.2	38.8	154	82.4	105	188	93.6	249	169	0.0	0.0	30.1
2003	9.6	58.3	129	155	43.4	230	262	115	121	11.3	7.7	30.6
2004	75.4	6.1	0.0	190	6.9	109	145	113	136	67.0	2.1	0.2
2005	27.0	7.0	94.0	221	267	166	395	169	275	134	29.8	0.0
2006	3.0	53.4	176	325	98.9	229	219	175	229	53.3	0.4	9.9
2007	5.6	185	67.0	91.3	116	NA	NA	NA	114	NA	NA	NA
2008	0.0	1.7	0.0	37.1	141	131	145	198	88.5	65.0	76.7	0.0
2009	35.5	4.5	23.8	31.0	42.3	26.3	187	68.1	34.0	52.2	0.0	0.0
2010	0.0	71.0	53.1	40.3	NA	142	68.2	140	71.2	23.2	8.8	4.5
2011	**0**	**0**	**51**	**49**	**49**	**149**	**218**	**180**	**86**	**0**	**14**	**0**
2012	**0**	**0**	**20**	**111**	**67**	**111**	**221**	**95**	**100**	**5**	**21**	**0**
						Temperature (T^0c)						
2000	17.7	19.0	20.6	20.7	19.7	18.2	17.6	17.3	18.6	18.3	19.1	19.4
2001	18.5	19.3	18.4	20.0	18.7	18.2	18.1	18.3	19.1	19.8	18.2	18.9
2002	17.9	18.4	19.6	19.8	19.7	18.8	18.6	17.8	18.6	19.6	19.2	18.5
2003	18.8	19.9	20.7	20.3	20.1	19.0	16.8	17.1	18.3	17.5	17.4	16.4
2004	18.2	18.1	18.6	20.0	19.7	19.4	19.1	18.6	17.5	17.1	16.9	17.4
2005	16.7	16.9	18.9	19.5	18.7	18.6	18.0	17.9	18.7	19.2	18.6	17.1
2006	17.9	19.2	19.4	18.8	18.8	19.1	18.5	NA	18.2	18.2	17.4	16.2
2007	19.2	19.1	20.1	18.8	19.1	NA	NA	NA	NA	NA	NA	NA
2008	17.8	19.0	19.4	20.3	20.5	18.5	17.5	17.3	18.1	18.0	16.4	16.0
2009	17.5	18.2	20.6	20.8	20.6	20.5	18.5	18.3	19.1	17.8	16.7	18.2
2010	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2011	**19**	**20**	**20**	**22**	**21**	**20**	**19**	**18**	**19**	**18**	**18**	**16**
2012	**17**	**18**	**19**	**19**	**19**	**18**	**17**	**16**	**17**	**18**	**19**	**17**

Source: Southern Nations, Nationality's Peoples Region (SNNPR) meteorological center. NA= Data Not Available

APPENDIX 4 Chromatogram from an internal standard of the EZ:faast method

Method Info : Test_Ezfaast_DUAL_GC-Method

Sample Info : PART#:7CG-G004-05 S/N 282507



Internal Standard Report

APENDIX 5

List of Amino acids analyzed by the EZ:faast method and their retention time.

Amino Acid	Retention Time
ALA	1.466
GLY	1.570
VAL	1.766
LEU	1.972
ILE	2.029
THR	2.234
SER	2.274
PRO	2.346
ASN	2.437
ASP	2.968
MET	2.999
HYP	3.132
GLU	3.314
PHE	3.345
GLN	3.935
LYS	4.577
HIS	4.763
TRP	5.332

APPENDIX 6

Mean values of some agronomic characteristics and their comparisons of fifteen chickpea cultivars tested under moisture treatment.

Cultivars	Biomass dry weight (g)		Seed weight plant^{-1}	Pod number plant^{-1}
	Control	Stress	Across moisture	Across moisture
Habru	6.69^{a}	4.49^{def}	4.16^{bc}	18.3^{bcd}
Mastewal	4.84^{de}	3.07^{ijk}	4.62^{abc}	19.7^{abc}
Landrace	4.30^{defgh}	2.62^{kl}	4.17^{bc}	25.1^{a}
Amit	4.99^{cde}	4.0^{efghij}	3.54^{cd}	17.6^{cd1}
CDC Chico	4.95^{cde}	3.56^{fghijk}	3.46^{cd}	20.2^{abc}
CDC Corinne	4.22^{defghi}	3.8^{efghijk}	4.88^{ab}	20.2^{abc}
CDC Leader	6.38^{ab}	4.88^{cde}	5.57^{a}	19.0^{bcd}
CDC Frontier	3.86^{efghij}	3.58^{fghijk}	1.59^{ef}	8.53^{ef}
CDC Orion	6.39^{ab}	4.21^{defghi}	5.0^{ab}	14.8^{cd}
CDC 820-32	3.18^{fghijk}	3.39^{fghijk}	4.88^{ab}	16.6^{cd}
ILC 3182	6.54^{a}	4.17^{defghi}	2.88^{de}	13.9^{de}
ILC 3279	4.53^{defg}	3.2^{hijk}	1.34^{f}	7.23^{f}
ILC 533	2.82^{jk}	1.73^{l}	4.02^{bcd}	23.3^{ab}
ILC 588	6.20^{abc}	3.23^{ghijk}	4.65^{abc}	15.5^{cd}
ICCV 2	5.29bcd	3.3fghijk	3.58cd	17.3cd
LSD	0.97		1.26	5.65
70 % FC	5.02^{a}		4.86^{a}	21.4^{a}
30 % FC	3.55^{b}		2.92^{b}	12.9^{b}
LSD	0.289		0.461	7.98

APPENDIX 7 Picture of chickpea root indicating size and number of nodules